ANNUAL REPORT
FIELD STUDIES AND STATISTICS PROGRAM
October 1, 1980 Through September 30, 1981

Dr. Joseph F. Fraumeni, Jr. continued this year as Acting Associate Director for Field Studies and Statistics (FS&S) in the Division of Cancer Cause and Prevention (DCCP). The program provides the focus for epidemiologic and biostatistical research within the Institute. It conducts intramural and collaborative epidemiologic investigation into the environmental and host determinants of human cancer; coordinates a network of population-based cancer registries for evaluating cancer incidence, mortality and incidence in the U.S.; analyzes the natural history of cancer and efficacy of therapeutic and preventive measures; and designs statistical models for clinical and experimental investigations. The organizational components are the Biometry Branch (Chief, Dr. Earl S. Pollack), the Clinical Epidemiology Branch (Chief, Dr. Robert W. Miller), and the Environmental Epidemiology Branch (Chief, Dr. Joseph F. Fraumeni, Jr.). In summary reports the Branch Chiefs have outlined the research activities under their direction. In this report the orientation and highlights of the FS&S program are briefly summarized and discussed in terms of new challenges and future directions.

Descriptive Epidemiology

A major objective of the FS&S program is to generate national statistics on cancer incidence, mortality, and survival. This provides valuable signals for further epidemiologic study, and for monitoring the progress of the National Cancer Program. The Surveillance, Epidemiology, and End Results (SEER) Program has collected population-based statistics on cancer incidence and patient survival since 1973, covering about 10% of the U.S. population. This year NCI Monograph No. 57 presents detailed data on cancer incidence and mortality for the period 1973-77, enabling a comparison of cancer incidence and mortality among various ethnic groups and geographic areas. A subcommittee of the Board of Scientific Counselors of DCCP recently completed a review of the SEER Program and recommended some changes to promote productivity, efficiency, and economy. Since non-melanoma skin cancer is not covered by the cancer registries, a special incidence survey was completed this year in various areas around the country. In the past a county-by-county survey of cancer mortality in the United States (1950-69) identified geographic peculiarities that have provided etiologic clues for further study, and this year an atlas was prepared on the geographic patterns of non-neoplastic diseases that may share etiologic factors with cancer. A series of special reports are planned to clarify the descriptive patterns of various cancers, utilizing the U.S. incidence and mortality data.

In the past, survival data were compiled on 450,000 patients diagnosed in 1950-73, based on data from four large cancer registries. This year the series was extended to include follow-up through 1978, permitting an analysis of trends in 5-year survival rates between those first diagnosed in 1970-73 and those in 1960-63. The survival rates increased significantly for most of the major sites of cancer. The SEER Program will soon provide follow-up data through 1980, so that 5-year survival rates can be evaluated for a large population-based sample of patients diagnosed in 1973-75. Major differences between whites and blacks have been found in the survival rates of various cancers, and studies are planned to identify the responsible factors.

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Analytical Epidemiology

Special emphasis was placed this year on case-control and cohort studies to evaluate key hypotheses in cancer etiology.

Occupational studies are a time-tested means of identifying physical and chemical carcinogens, and were pursued to assess hazards suspected on the basis of experimental, clinical, and field observations. Preliminary surveys of petroleum workers revealed excesses of certain cancers, notably of the brain, while professional artists showed increases of leukemia and bladder cancer. Considerable effort was devoted to working with other Government agencies such as the Social Security Administration, to utilize record systems that could help in identifying occupational groups at high risk of cancer.

Radiation studies received more emphasis as pressure mounts to clarify the effects of low-level exposure and the shape of the dose-response curve. To characterize the risk of radiogenic breast cancer, parallel analysis was conducted on three major sets of human data (tuberculosis patients receiving multiple fluoroscopic exams of the chest, mastitis patients given radiotherapy, and atomic bomb survivors). The findings suggested that the risk of breast cancer is greatest in persons exposed as adolescents, although exposure at older ages also carries some risks; the dose-effect relationship is consistent with linearity; fractionation does not appear to diminish risk nor does time since exposure, even after 45 years of observation; and the interval between exposure and clinical appearance of radiogenic breast cancer is mediated by age-related factors (hormonal?).

Drug studies were expanded to evaluate the effects of estrogenic compounds, immunosuppressive and cytotoxic agents, and other medications suspected to have carcinogenic activity. A relationship was found between the use of menopausal estrogens and the risk of breast cancer. However, no major excess of breast cancer was associated with use of oral contraceptives, although some groups of women (e.g., with familial susceptibility or benign breast disease) appear predisposed to the effects of these agents.

Nutritional studies were intensified this year to clarify the role of dietary fat, fiber, micronutrients, and food additives in cancer etiology. Field studies are taking advantage of geographic areas of the U.S. (e.g., North/South differentials for colorectal cancer) and migrant groups (e.g., Japanese- and Norwegian-Americans) whose cancer risk may be altered by changing dietary habits. To evaluate the relationship between bladder cancer and saccharin, a case-control interview study of 3,000 cases and 6,000 controls revealed no overall excess risk for persons who had ever used artificial sweeteners. However, the findings among very heavy users were consistent with experimental studies suggesting that saccharin is a weak carcinogen by itself or may promote the effects of other carcinogens.

Family studies, enhanced by the development of a computer-based data resource, resulted in the delineation of family cancer syndromes and mechanisms of host susceptibility. Of special interest was the identification of the dysplastic nevus syndrome as a specific marker of susceptibility to melanoma. Recognition and proper management of these moles enables the primary prevention of melanoma and the diagnosis of this potentially lethal cancer at a stage when it is readily curable. Educational videotapes were developed to

acquaint high-risk patients and health professionals with the specifics of dysplastic nevi and the opportunities for prevention and early detection of melanoma. In addition, studies of neurofibromatosis have helped to clarify the risks of various cancers associated with a hereditary syndrome that has received widespread public attention in recent times.

Environmental pollutants were evaluated through epidemiologic studies that integrate appropriate environmental and body measurements. Nearing completion are investigations to evaluate the relation of bladder cancer to trihalomethanes in drinking water, and the association of lung cancer to industrial emissions of arsenic into the general community.

Case-control studies of selected cancers have been carried out when highrisk communities are identified on the cancer maps, or when major testable hypotheses and special resources become available. Based on leads provided by the cancer maps, field studies have implicated shipyard work during World War II as the explanation for the high rates of lung cancer in coastal areas, snuff dipping (smokeless tobacco) for the high rates of mouth cancer among females in the southern United States, and nutritional deficiencies and alcohol consumption for the high rates of esophageal cancer among men in urban areas.

Multidisciplinary projects combining epidemiologic and experimental approaches were given special emphasis to evaluate the influence of oncogenic viruses, dietary and metabolic factors, host susceptibility, air and water pollutants, and other causative factors that may continue to elude detection by traditional observational methods.

Collaboration

Collaborative studies with other Federal agencies have been given high priority to (1) evaluate urgent issues including those of immediate regulatory or public policy concern, and (2) to stimulate the epidemiologic application of technical and data resources that are used by the Government mainly for other purposes. Many research and regulatory agencies are concerned with environmental cancer, yet few have epidemiologic programs, and require assistance and support on many issues. Particularly at this time of fiscal restraint, it is important to increase initiatives to develop and coordinate national data resources that, with appropriate safeguards, may be tapped by qualified investigators throughout the country. FS&S staff are also working closely with other groups in efforts to remove the serious obstacles to epidemiologic research resulting from extension of the privacy and confidentiality laws and by the excessive delays and complexities created by clearance procedures. The bi-national programs offer major epidemiologic opportunities for international study, and this year special emphasis was given to joint studies and exchange programs with Chinese scientists to pursue clues drawn from the recent county-based maps in China and the changing risks among Chinese migrants to the United States. Within the Institute, further steps are being taken to improve communication and coordination of epidemiology and biometry programs, and to stimulate multidisciplinary activities linking epidemiologists with experimentalists and clinicians.

Biometrics

The development of statistical methodology in FS&S has contributed greatly to several areas, including epidemiology, carcinogenesis research, therapy trials, and screening programs. This year attention was given to the development and testing of multi-cause and multi-stage models of carcinogenesis, and the clarification of issues involved in extrapolating results from animal experiments to man. There was substantial involvement in the study design, implementation, and analysis of therapy trials for various forms of cancer. The FS&S program continued to be responsible for statistical support and consultation to intramural scientists throughout the Institute, ranging from basic laboratory research to community activities in cancer control. Further emphasis was given to the development of new statistical techniques in designing, monitoring, and evaluating programs for the screening and early detection of cancer. With the development of a new focus for screening programs in the Division of Resources, Centers and Community Activities (DRCCA), it will be important to establish a close working relationship between appropriate staff in DRCCA and DCCP.

Prospects

It is difficult to project activities over time, given the uncertainties related to reorganization plans, available positions, funds, manpower, and especially to the direction that new leads and opportunities will take. However, a major objective of FS&S is to attain a comprehensive, flexible, and balanced program that will enhance our capacity at the national level to generate fresh ideas and help settle key questions in cancer epidemiology and biometry. Toward this end, a selective expansion and reorientation of the program seems warranted to augment existing project areas, initiate new lines of research, and make the most efficient use of resources located at NCI and several Federal agencies. Although its primary responsibility is intramural research, FS&S has a clear obligation to provide biometric and epidemiologic support to all parts of the National Cancer Program, to foster parallel efforts throughout the Program, and to promote epidemiology training opportunities at NIH and elsewhere. With mounting interest in environmental cancer and in the contribution to etiology and prevention that can be made through the epidemiologic approach, FS&S has been asked to increase the scope of its work and to help develop Federal programs and policy in several areas. Despite substantial growth and support of the epidemiology program over the past four years, there are still insufficient senior staff to keep pace with the opportunities and demands for research and consultation in the field of environmental cancer. If funds and personnel become available, special priority would be given to research designed to clarify the role of nutritional factors and general environmental pollutants in cancer etiology. It should be emphasized that the FS&S program contributes not only to cancer etiology, but also to natural history, end results, clinical trials, preventive measures, and even strategies involved in administrative planning and decision-making. Epidemiologic and biometric approaches permeate many aspects of the National Cancer Program and are fundamental to the design and evaluation of methods to control cancer. The effectiveness of FS&S thus depends greatly upon the degree of interaction and coordination with other parts of the Institute.

ANNUAL REPORT
BIOMETRY BRANCH
October 1, 1980 through September 30, 1981

The major functions of the Biometry Branch are: to measure trends in cancer incidence and patient survival over time and to assess differences in these measures among important population subgroups; to conduct research on the etiology of cancer in humans; to develop statistical methodology applicable to clinical trials and other follow-up studies as well as to other problems in cancer research; and to provide statistical and computer science support to other research investigators outside the Branch. This work is accomplished through in-house studies and through field studies, some of which are carried out collaboratively with investigators in this country and abroad. The program is summarized briefly as follows:

Surveillance, Epidemiology and End Results (SEER) Program

The SEER Program obtains cancer incidence and patient survival data in the United States through ten population-based cancer registries covering all cancers diagnosed in the populations of five entire states (Connecticut, Hawaii, Iowa, New Mexico and Utah), four metropolitan areas (Atlanta, Detroit, San Francisco and Seattle), and the Commonwealth of Puerto Rico. The Program is operated primarily by Dr. John Young and his Demographic Analysis Section. Because of the size and complexity of this program, it has come under considerable scrutiny by the review groups, the most recent of which was a review carried out by a subcommittee of the Board of Scientific Counselors of DCCP. The primary thrust of the recommendations of this review group was that the program should focus on the cancer incidence aspect and relegate to secondary status the measurement and analysis of cancer patient survival and the use of the program as a mechanism for carrying out related epidemiologic studies. As a result of this, some substantial modifications of the program are now being planned and will be implemented over the next few months. One of the major purposes of these modifications, aside from responding to the recommendations of the Board of Scientific Counselors, is to bring about a fairly substantial reduction in cost. At the same time, plans are being made to increase the program's coverage of black and Hispanic populations in the United States, areas in which coverage of the program have been deficient. Part of this deficiency was due to the necessity of discontinuing the inclusion of the New Orleans metropolitan area in the SEER Program. This has changed somewhat the geographic balance and also has created a further undercoverage of the black population.

NCI Monograph No. 57, presenting cancer incidence and mortality data for 1973-1977 for the eleven participants included in the program at that time, is scheduled for a June 1981 publication date. Included in this publication are some detailed data according to specific ethnic groups. Striking differences occurred among race-sex groups in geographic areas with respect to the incidence of cancer of various sites. Examples of these differences are as follows:

Stomach: The incidence of stomach cancer was high among Japanese and Hispanics and among white populations with high percentages of foreign-born persons (Detroit, San Francisco-Oakland). The incidence was higher among blacks than among whites for both sexes.

Colon: Although the risk of colon cancer was similar between males and females for whites and blacks, males had slightly higher rates. The highest incidence was reported for Connecticut and the lowest for New Mexico, Utah and Puerto Rico.

Rectum: The pattern for rectal cancer was similar to that for colon, although rates for blacks were lower than those for whites. Again, the highest incidence occurred in Connecticut and the lowest in New Mexico, Utah and Puerto Rico.

Lung: Blacks and Hawaiians of both sexes experienced higher incidence of lung cancer than did whites. Extremely high rates were reported for New Orleans whereas those for Utah were the lowest. Rates among males almost quadrupled those for females.

Breast: Breast cancer was the most frequent female cancer in each race/ethnic group. The highest incidence was reported among Hawaiian and white females. Among whites, the highest incidence was reported in Hawaii and the lowest incidence in Utah. Rates for Hispanic females were approximately one-half those for other whites.

Cervix: The risk of invasive cervical cancer was much higher among the American Indians, blacks, Hawaiians, and Hispanics than among other whites. A similar disparity was noted for in situ carcinomas of the cervix among blacks and American Indians as compared to whites.

Corpus Uteri: Both white and Hawaiian females experienced a high risk of cancer of the corpus uteri. A major exception occurred in New Orleans among white females who had the lowest rates for whites among the geographic areas. Rates among whites residing in the Pacific region (Seattle-Puget Sound, San Francisco-Oakland, and Hawaii) were much higher than those reported for other areas.

Prostate: The incidence of prostate cancer was much higher among blacks than among any other race/ethnic group. The highest rates for whites were reported for Hawaii, Utah, and Seattle-Puget Sound.

Bladder: The risk of bladder cancer was three to four times higher among males than among females. The highest rates, for white males, who experienced rates almost double those for any other race/ethnic group, were reported by Hawaii and New Orleans; the lowest rates occurred in Utah.

Preliminary examination of the cancer incidence rates for 1978 and 1979 reveal that for the first time since cancer incidence data had been available (1935) black females had higher rates than white females (305.8 for black females versus 295.3 for white females compared with 295.4 versus 302.0 in 1978). This may be an artifact, however, since this excess rate for black females occurred only in Detroit, one of the three areas with significant black populations, the other two being Atlanta and San Francisco. Since there is some question about the validity of the population estimates used as denominators for these rates for the black population, a definitive answer will not be forthcoming until the results of the 1980 census by age, race and sex for each area become available.

With respect to survival data, tapes will be submitted to NCI in September 1981 containing follow-up on all patients diagnosed 1973-79 and followed at least through December 1980. This will allow the calculation of five-year survival rates for patients diagnosed 1973-1975, three-year survival rates for patients diagnosed 1973-1977 and one-year survival rates for patients diagnosed 1973-1979.

Clinical and Diagnostic Trials

Dr. Byar and his staff perform two major important functions -- consultation on the development of large clinical trials and the development of statistical methodology for the analysis of data resulting from such trials and from related studies. In consultations on clinical trials, members of the Section assist investigators in developing detailed study protocols, in determining numbers of patients necessary for the study, and deciding what data should be recorded and at what intervals in time, and in developing forms for the recording of data. They provide advice on the proper methods of analysis of the data resulting from the trials or undertake these analyses themselves. During the year this group continued their involvement in major clinical trials for the following cancers: prostate, bladder, testis, breast, lung, brain, and mycosis fungoides. In addition, they pursued a number of areas of investigation into statistical methodology and several papers have resulted from this work. The following is a brief summary of some of these activities during the year.

1) Based on data obtained from randomized clinical trials on prostate cancer conducted by the Veterans Administration Cooperative Urological Research Group, the final report was published comparing prostatectomy plus placebo versus placebo alone for patients with stage I and II prostatic cancer. The results of the study suggest that cancer of the prostate, like breast cancer, metastasizes early in some patients and that some systemic therapy should be used in addition to, or instead of, surgery.

2) Assistance is being provided to the Genito-Urinary Group of the European Organization for Research on Cancer comparing pyridoxine plus placebo in the treatment of bladder cancer. Patients are still being accessioned and analyses of the data are being carried out periodically.

3) A nationwide randomized trial comparing adjuvant combination chemotherapy following surgery for resectible stage II testicular cancer versus chemotherapy only for relapses is still underway. The staff of Dr. Byar's section is responsible for the study design, implementation and analysis.

4) Serum and background data have been collected from nearly 10,000 women for evaluation of biological markers for breast cancer in cooperation with the Markers Group of the Breast Cancer Task Force. Dr. Byar's section is responsible for the collection, editing and analysis of all of the data and for providing a continually updated inventory of materials in the Serum Bank.

5) Two staff members are serving as statisticians for the Lung Cancer Study Group, which is comprised of six major centers with a capacity to recruit over 150 stage I lung cancer patients per year. Five prospective

randomized trials are in progress. In addition, the staff is surveying reasons for non-entry into the present trials in an effort to improve accrual and is studying characteristics of the available patient population for accrual into future trials.

6) Work has continued on the design and analysis of a number of large-scale randomized clinical trials in collaboration with the Brain Tumor Study Group. Planning and design work is also being carried out for two new clinical trials -- a phase III trial to investigate combined and sequential chemotherapy and a phase II trial of new chemotherapeutic agents.

This group is involved in a number of additional projects including an evaluation of hospice care for terminal cancer patients, studies of the Makari Skin Test to evaluate it as an aid in diagnosis and to determine the prognostic value of the test in predicting subsequent recurrence after surgical resection for colorectal, lung, and breast cancer, analysis of records on a large number of patients with Burkitt's lymphoma who were treated in Ghana to assess the prognostic value of a simple staging system, and a number of other studies.

This section has been very active in the development of statistical methodology related to various aspects of their work. The following is simply a list of topics of this research to indicate the range of this activity: sequential monitoring of clinical trials, accrual stopping rules for clinical trials, analysis of time-dependent covariates, adjustment of survival curves for differences in patient covariates, power computations for comparative Poisson trials, subset analyses in epidemiologic studies, likelihood calculations for matched case-control studies and survival studies with tied death times, confidence limits for estimates of median survival time, non-parametric estimation for a scale-change model with censored observations, testing symmetry and independence in a bivariate distribution function with incomplete paired data, and several others. In addition, new programs have been added to an interactive computer system for analyzing clinical trial data and data for similar types of studies.

Mathematical Statistics

Dr. John Gart and his Mathematical Statistics and Applied Mathematics Section has continued to provide statistical support and consultation to intramural scientists within the Division of Cancer Cause and Prevention as well as to those in other divisions in NCI. This involves basic study design as well as data analysis. In addition, this section continues to develop basic statistical methodology with applications to the kinds of problems involved in their consultative work but with broader applications as well.

A brief summary of some of the activities of this section during the past year is as follows:

1) Statistical analysis is being carried out on two large prospective studies on the relationship between diet and cancer -- one in Minnesota and the other in Norway. One of the papers resulting from these analyses documents the negative association between lung cancer and vitamin A consumption,

while a second paper investigates the association of cancer at several sites with the use of alcohol. Pancreatic cancer appears to be associated with alcohol use.

2) A number of collaborative studies have been carried out in the area of carcinogenesis research. These include mammalian cell transformation assays to study the combined effect of mixtures of low doses of known mutagens; study of the effects of fractionated doses of the synergism between radiation and estrogen administration in mammary carcinogenesis; a study examining the survival of lymphoid cells from ataxia telangectasia patients and their related heterozygotes after exposure to bleomycin; the design and analysis of factorial experiments to study the factors which influence the growth of human cells in culture; and a number of others.

3) Several collaborative efforts have been carried out with the Division of Cancer Biology and Diagnosis. They include a case-control study of the association between Dermatitis Herpetiformis disease and the Second B cell antigen (SB) locus of the HL-A system; a study of the in vitro survival of lymphocyte and fibroblast cell lines from patients with cancer prone disease, xeroderma pigmentosum, and other hereditary degenerations after exposure to DNA-damaging agents; and several others.

4) Extensive research has been carried out on a broad spectrum of topics in mathematical statistics, probability, and applied mathematics. This includes an evaluation of three methods for approximating confidence limits for the odds ratio; an empirical Bayes method of incorporating historical control data into tests for trends in proportions; the development of simple estimators of summary relative risk for stratified data from prospective studies; development of simple estimators of hazard ratios for censored survival data; research in statistical genetics on generalized ABO-systems; an investigation of the properties of the relative risk for case-control studies with multiple matched controls; an investigation of higher order corrections to the mean and higher order moments of various transformations of binomial proportions; and a number of other developments.

Analytic Studies

Dr. Max Myers and his Biometrics Research and Analytic Studies Section conduct research in cancer etiology, screening for early detection, prognosis, and in statistical methodology related to each of these areas. Some of this work is carried out through the use of data from the Surveillance, Epidemiology and End Results (SEER) Program. Others involve the use of data from other sources, while still others require study design and collection of data from specific populations. Some of the activities of this group over the past year include:

1) Work has begun on a large collaborative study involving five institutions in collaboration with the Clinical Epidemiology Branch to assess morbidity among long-term survivors of childhood cancer and their offspring. Study design has been completed and interviews are being conducted among these survivors and their siblings as controls.

2) Recently published results on cancer patient survival have indicated that white cancer patients had a more favorable survival experience than blacks, part of which was due to a more favorable stage distribution and diagnosis. Largest differences in survival between whites and blacks occurred for cancer of the urinary bladder for each sex and for cancer of the uterine corpus.

3) Work has continued on study of multiple primary cancers using historical data from the Connecticut Tumor Registry. A computer system has been completed to permit rapid analysis of the subsequent cancer experience of patients with specific primary cancers.

4) An intensive study of the relationship between occupation and cancer of the lower urinary tract was carried out in Detroit through a population-based case-control study conducted as part of the National Bladder Cancer Study. The findings suggested that truck drivers have a significantly increased risk of lower urinary tract cancer with the risk increasing with increased duration of employment as a truck driver.

5) A comparison of the survival experience on patients diagnosed with non-seminomatous testicular cancer during the period 1973-75 with those diagnosed between 1976-79 revealed a substantial improvement in survival. This supports the suggestions that improved combination chemotherapy for this cancer has been introduced on a broad scale since 1975.

6) Detailed analysis of cancer staging has been carried out for stomach cancer using almost 3,000 SEER patients diagnosed in 1977-78 with survival recorded through 1979. A similar analysis was carried out for colon cancer. The results seem to indicate that the detailed SEER extent of disease information is compatible with the system of staging developed by the American Joint Committee.

7) The interactive computer system developed by the Clinical and Diagnostic Trials Section has been modified to permit its application to datasets as large as 10,000 records. This has proven to be a powerful analytic tool for a number of the analyses carried out by the Biometric Research and Analytic Studies Section as well as by others in the Biometry Branch.

8) Work has continued on the development of theory for the evaluation of screening programs for the early detection of cancer using methodology from renewal theory. Topics being investigated include age dependence, parameter estimation, leadtime, length-biased sampling and mortality measures.

International Studies

Dr. Tu Ji-Tao, an epidemiologist from the Shanghai Cancer Registry, is spending a year with the Branch as a Visiting Fellow. He brought with him the cancer incidence data from the Shanghai Registry for the period 1972-1978. After the Shanghai incidence rates were standardized to the 1970 U.S. population, they were compared with those for the Chinese populations in San Francisco and Hawaii. There were extraordinary excesses in cancer risk in

Shanghai for cancers of the stomach, esophagus, liver and cervix and extremely low rates for cancers of the uterine corpus and prostate. Further analyses of these comparisons are being carried out and the possibility of collaborative studies in the Chinese populations in San Francisco and Shanghai are being explored.

Since the cancer mortality rates for the People's Republic of China have become available, two members of the staff have carried out detailed analyses of these rates in comparison with those for Chinese populations in the U.S. These analyses have revealed sharp reductions in the mortality risk among Chinese migrants to the United States for stomach, esophagus and liver cancers and substantial increases for lung and colon cancers.

Skin Cancer

Joseph Scotto has been directing an extensive study of the incidence of non-melanoma skin cancer in the United States. Preliminary findings suggest that among caucasians in the United States, the annual incidence of non-melanoma skin cancer may be 400,000, about one-half the number for all other cancers combined. A major interest in the study of these cancers has been on the possible impact of ultraviolet radiation on the occurrence of these cancers. During this year, mathematical models have been applied to a new set of data using eight new locations and two old locations to examine the relationship between ultraviolet radiation and the occurrence of skin cancer. Estimates from these models indicate that a one percent increase in ultraviolet radiation may result in slightly less than two percent increase in skin cancer. This implies that stratospheric ozone reductions of one percent may eventually result in a four percent increase in skin cancer. With large decreases in stratospheric ozone (over ten percent), the subsequent increases in skin cancer may be even greater than four-fold.

Computer Science

During this year, Mr. Theodore Weiss left the position as Chief of the Computer Science Section to take another position and Mr. J. Michael Stump has been serving as acting Chief of that section. The section has continued to monitor a large computer support contract to provide computer systems and programming support to various projects within the Biometry Branch. The section has also continued its heavy involvement in the development of design and software for computer systems for some of the large cancer registries in the SEER Program. A number of components of the new computer system for the Connecticut Tumor Registry have been completed during the year and it is anticipated that the total system will be ready for a thorough test by the end of 1981.

Plans for the Immediate Future

The action of the Board of Scientific Counselors relative to the SEER Program was mentioned above. Their specific recommendations included reduction of active follow-up of patients in the SEER registries to a minimum; reduction

in the coding of extent of disease; reduction to a bare minimum of the amount of SEER contrac. funds devoted to epidemiologic research; exploration of existing non-SEER registries for the possibility of utilizing their data in conjunction with those from SEER, and implementation of sound cost accounting procedures throughout the SEER Program. The staff will take these recommendations seriously. A study throughout the SEER Program of the feasibility of converting to passive follow-up will be carried out immediately and if this proves to be feasible, passive follow-up will begin in 1982. Changes will also be made in relation to the other recommendations with a view toward a substantial reduction in the total cost of the program. Within this framework, exploration will be completed for the addition of one or two new registries to the program to provide more adequate coverage of the black and Hispanic populations.

A study of the incidence and mortality of stomach and colon cancer among Puerto Ricans in New York City will be initiated in the Fall of 1981. When the data resulting from this study are compared with those from the Commonwealth of Puerto Rico, already obtained through the SEER Program, it should be possible to determine whether findings from earlier mortality studies -- that colon cancer rates among Puerto Rican migrants to New York City do not appear to increase -- are artifacts or whether indeed this phenomenon operates differently from that in other migrant populations.

We will investigate further the possibility of developing collaborative studies with the cancer epidemiologists in Shanghai to identify factors associated with the vast differences in cancer incidence rates between the Shanghai population and the Chinese populations in the United States. Technical assistance will also be provided to the Shanghai Cancer Registry to enable them to more readily process the large volume of data in order to carry out systematic analyses.

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October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Cancer Surveillance, Epidemiology, and End Results Reporting (SEER) Program

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.: Earl S. Pollack, Chief BB NCI
John L. Young, Jr., Chief, Demographic Analysis Section BB NCI

Other: See attached sheet

COOPERATING UNITS (if any)

See attached sheet

LAB/BRANCH
Biometry Branch, Field Studies and Services Program

SECTION
Demographic Analysis/Biometric Research & Analytic Studies/Computer Science

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Data on cancer patients diagnosed from year of entry into the SEER Program through 1979 (1973-79 for most participants) were submitted to the NCI by the ten participants in December 1980. Analysis of cancer incidence revealed considerable variability across all areas and among the various ethnic groups represented therein. Cancer mortality rates for whites for the total SEER Program are virtually identical with those for the entire U.S. Survival rates for some of the major sites of cancer also reveal differences among the various areas. Incidence data from the first five years have been published in NCI Monograph 57.

Other Professional Personnel:

Sandra Abbott, Statistician	BB	NCI
Ardyce J. Asire, Statistician (Health)	BB	NCI
Betty J. Cicero, Medical Record Librarian	BB	NCI
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Jack Cunningham, Medical Record Librarian	BB	NCI
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Cooperating Units:

California Department of Health Services
University of California, San Francisco
Connecticut Department of Health
Fred Hutchinson Cancer Research Center, Seattle
Research Corporation of the University of Hawaii
University of Iowa
Michigan Cancer Foundation
University of New Mexico
University of Utah
Yale University
Emory University
Geomet, Incorporated
Commonwealth of Puerto Rico Department of Health

Project Description

Objectives:

To provide descriptive data on cancer incidence and patient survival for defined populations; to investigate variation in risk of specific forms of cancer by region, place of residence, age, sex, race/ethnicity, socioeconomic status. Based on analyses of differences among subgroups, to develop hypotheses concerning etiology for further study; to evaluate patient survival for trends over time, taking into account changes in the distribution of extent of disease and type of treatment, and to compare survival among areas and among population subgroups to identify variables that might be related to variation in survival rates. To promote specialty training in epidemiology, biostatistics, and tumor registry methodology, operation and management.

Methods Employed:

The incidence and survival data are obtained from a group of ten population-based cancer registries covering all cancers diagnosed in the populations of five entire states (Connecticut, Hawaii, Iowa, New Mexico, and Utah), four metropolitan areas (Atlanta, Detroit, San Francisco, and Seattle) and the Commonwealth of Puerto Rico. Data were collected previously from New Orleans, Louisiana but the program has been discontinued in that area. For each case of cancer diagnosed in these areas, information is obtained from hospital records on age, sex, race/ethnicity, place of residence, site of cancer, histologic type, extent of disease, type of treatment. Each alive case is followed at least once a year after diagnosis or date of last contact to determine vital status. A complete updated tape is submitted to NCI each year from each registry; it contains data on all cases diagnosed during the preceding year and follow-up data on all previously reported cases for the latest year.

In order to assure complete, accurate and comparable data among the population-based registries supplying data to the SEER Program, an extensive program of training and quality control procedures is employed. Training is carried out in a variety of ways depending on the need and those being trained. Periodic workshops, attended by representatives from all areas, are conducted to introduce new procedures or to reinforce existing data abstracting and coding rules and conventions. Other workshops are conducted at a local level to train new employees or to upgrade the level of abstracting by hospital tumor registrars who contribute to the central registries. Completeness of reporting audits consists of matching of cases identified from a variety of sources, such as pathology lists, radiotherapy rosters, autopsy reports, oncology department patient files, etc., against the registry file to determine completeness of case finding. Accuracy and comparability are evaluated by reabstracting and/or recoding a sample of the cases, and checking these against the codes on file. Differences are investigated and corrective procedures instituted. Performance reports are given to each participant.

Extensive use of computer edit programs are employed on the data submitted to NCI. This includes a check to ensure that all codes are within the bounds specified. Items within the abstract are compared, such as a date of birth with age, sex with specific site, site with histologic type, etc. For patients who had more than a single cancer, items between extracts are compared to ensure consistency. These edit programs produce printouts, presenting the entire case as well as a message as to the item in question, which are sent back to the registry for resolution.

The registries serve as a basis for research projects conducted by the staff of the individual registries. Once these projects are designed, they are usually carried out through funds obtained from sources other than the basic SEER contract. Since the registries provide complete coverage of all cancers in defined populations, they are an ideal resource for carrying out collaborative case-control studies to test the impact of suspected etiologic factors for specific forms of cancers. NCI-initiated studies are carried out by the Biometry Branch or the Environmental Epidemiology Branch through contracts with the SEER registries.

In addition, mortality data are obtained from the National Center for Health Statistics (NCHS) so that cancer incidence and mortality rates for the SEER areas can be compared and so that cancer mortality rates for the SEER areas can be compared to those for the United States as a whole. Mortality data for the Commonwealth of Puerto Rico are not available through the NCHS but must be obtained directly from the Department of Health in Puerto Rico. Normally, NCHS releases mortality data for the previous calendar year in late October. However, this year mortality data for 1978 did not become available in machine readable form until late April 1981, and there is every reason to think that similar delays will continue into the future. Thus, incidence data for a given calendar year will be available for analysis one to two years prior to mortality data.

Population estimates of the various geographic areas are obtained from a variety of sources. Estimates by five-year age groups for whites are available for every county in the United States through 1978. However, complicated statistical procedures must be applied to estimate population for Hispanics, blacks, Chinese, Japanese, Filipinos, Hawaiians and American Indians. The accuracy of these procedures diminishes the further the year from the 1970 census. Corrections to population estimates can be applied once data from the 1980 census are released. However, every indication is that these data will not be available until well into the next fiscal year. In the interim, incidence and mortality rates for 1978 forward for races other than whites should be interpreted with extreme caution.

Major Findings:

Data for cases diagnosed between January 1, 1973 and December 31, 1979 were submitted to NCI in December 1980. One participant submitted data only for 1974-1978; one submitted data only for 1975-1978. While it is felt that all areas have

complete reporting for 1978, data for 1979 appear to be incomplete. In addition, survival data for patients diagnosed between January 1, 1973 and December 31, 1978 are available through at least December 31, 1979. There was considerable variation among the participants with respect to the percent of patients not known to be dead who were actually followed into calendar year 1979 or 1980. Three of the ten participants have rates which are unacceptably low and major efforts to improve this deficiency are underway.

Incidence and mortality data for 1973-1977 for the eleven participants included in the program at that time have been published in NCI Monograph 57. A synopsis of the major findings of those data follows:

The average annual age-adjusted incidence rate for all forms of malignant neoplasms for all races and both sexes combined for all SEER areas except Puerto Rico was 331.5, whereas the rate for Puerto Rico was 200.4. Among the other areas, rates ranged from a low of 279.8 in Utah to a high of 363.5 in San Francisco-Oakland.

The average annual rate of carcinoma in situ for all SEER areas excluding Puerto Rico was 26.7; the rate for Puerto Rico was 16.9. Among the other areas, these rates ranged from a low of 19.2 in Utah to a high of 41.8 in Atlanta.

The most common primary sites of cancer were colon, rectum, breast, and lung with age-adjusted rates of 48.5, 46.7, and 46.7, respectively. In fact, these 3 sites accounted for 42.7% of all malignant cancers. Seventy-eight percent of the in situ lesions occurred in the female genital system.

The average annual mortality rate due to malignant neoplasms for all SEER areas, excluding Puerto Rico, was 168.5 compared with a rate of 166.5 for the total United States. The comparable rate for Puerto Rico was 127.0. Rates ranged from a low of 123.6 in Utah to a high of 201.1 in New Orleans. The mortality rate for Puerto Rico was slightly higher than that for Utah, even though the incidence rate was considerably lower (200.4 vs. 279.8). This difference reflects the higher proportion among Puerto Rican patients with cancer at primary sites for which survival is poor (esophagus, stomach, liver) than in patients in Utah. Similarly, the high mortality rate in New Orleans is reflective of the high incidence of lung cancer in that area.

Of all cancer deaths, lung cancers accounted for 21.7%; colorectal, 13.6%; and breast cancers, 9.2%. These 3 sites accounted for over 40% of cancer cases and deaths, although proportionately more deaths were attributable to lung cancer.

Considerable variation is noted in both incidence of and mortality from cancer among the various racial and ethnic groups for which data are available. The following list shows the variation in average annual age-adjusted incidence rates for all sites combined among the races for each sex:

Males

Hawaiian, Hawaii (465.0)
Black, all areas (454.3)
White, all areas (371.6)
Japanese, Hawaii (327.6)
Chinese, San Francisco-Oakland (325.6)
Chinese, Hawaii (262.9)
Filipino, Hawaii (249.5)
Hispanic, New Mexico (229.5)
Hispanic, Puerto Rico (229.2)
Japanese, San Francisco-Oakland (222.0)
American Indian, New Mexico (178.4)

Females

Hawaiian, Hawaii (408.5)
White, all areas (301.2)
Black, all areas (288.7)
Chinese, San Francisco-Oakland (283.6)
Chinese, Hawaii (263.0)
Hispanic, New Mexico (237.1)
Japanese, San Francisco-Oakland (224.0)
Japanese, Hawaii (220.9)
American Indian, New Mexico (191.6)
Filipino, Hawaii (191.5)
Hispanic, Puerto Rico (173.6)

In general, the highest rates occurred among Hawaiians, blacks, and whites. Among males, blacks had higher rates than whites, but the reverse was true among females. The contrast in rates among the Chinese and Japanese in Hawaii versus those in San Francisco-Oakland is striking. Both Chinese and Japanese females residing in these 2 areas experienced similar rates. However, wide disparity was noted among males; the Japanese in Hawaii had much higher rates than those in San Francisco-Oakland, whereas Chinese males residing in San Francisco-Oakland had much higher rates than those in Hawaii. Although Hispanic males in New Mexico had rates almost identical to those in Puerto Rico, Hispanic females residing in New Mexico had much higher rates than those in Puerto Rico.

Although, generally, males experienced higher rates than females, male and female Japanese residing in San Francisco-Oakland had equal rates; but Hispanic and American Indian males residing in New Mexico had lower rates than those experienced by females.

Striking differences occurred among the race-sex groups and geographic areas with respect to the incidence of cancer of various sites. A few of these differences were:

Stomach: The incidence of stomach cancer was high among Japanese and Hispanics and among white populations with high percentages of foreign-born persons (Detroit, San Francisco-Oakland). The incidence was higher among blacks than among whites for both sexes.

Colon: Although the risk of colon cancer was similar between males and females for whites and blacks, males had slightly higher rates. The highest incidence was reported for Connecticut and the lowest in New Mexico, Utah, and Puerto Rico.

Rectum: The pattern for rectal cancer was similar to that of the colon although rates for blacks were lower than those for whites. Again, the highest incidence occurred in Connecticut and the lowest in New Mexico, Utah, and Puerto Rico.

Lung: Blacks and Hawaiians of both sexes experienced higher incidence of lung cancer than did whites. Extremely high rates were reported for New Orleans, whereas those for Utah were the lowest. Rates among males almost quadrupled those for females.

Breast: Breast cancer was the most frequent female cancer in each race/ethnic group. The highest incidence was reported among Hawaiian and white females. Among whites, the highest incidence was reported in Hawaii, the lowest in Utah. Rates for Hispanic females were approximately one-half those for other whites.

Cervix: The risk of invasive cervical cancer was much higher among American Indians, blacks, Hawaiians, and Hispanics than among other whites. A similar disparity was noted for *in situ* carcinomas of the cervix among blacks and American Indians as compared with whites.

Corpus uteri: Both white and Hawaiian females experienced a high risk of cancer of the corpus uteri. A major exception occurred among the white females in New Orleans who had the lowest rates for whites among the geographic areas. Rates among whites residing in the Pacific region (Seattle-Puget Sound, San Francisco-Oakland, Hawaii) were much higher than those reported for other areas.

Prostate: The incidence of prostate cancer was much higher among blacks than among any other race/ethnic group. The highest rates for whites were reported for Hawaii, Utah, and Seattle-Puget Sound.

Bladder: The risk of bladder cancer was three to four times higher among males than among females. The highest rates for white males, who experienced rates almost double those for any other race/ethnic group, were reported by Hawaii and New Orleans; the lowest occurred in Utah.

Within primary site groups there were several major shifts among histologic cell types involved when data for 1973-77 were compared to comparable data available from the Third National Cancer Survey (TNCS) 1969-71. Six of these differences were:

1. A large increase has occurred in the relative frequency of malignant carcinoids of the small bowel and a minimal increase in carcinoids of the ascending colon.

2. The relative frequency of osteosarcomas had decreased from TNCS to SEER (44% vs. 34%, respectively) and has been offset to a certain extent by a rise in chondrosarcoma from 23 to 28% and, to a lesser extent, by a slight increase in the relative frequency of chordomas and giant cell tumors of the bone.

3. Malignant fibrous histiocytoma has become the third most frequent histologic type of soft tissue tumors.

4. The relative frequency of adenosquamous carcinomas of the cervix and corpus has increased considerably.

5. Endometrioid carcinoma has emerged as an important type of ovarian cancer.

6. The relative frequency of mixed tumors of the salivary gland has decreased as has the incidence of salivary gland cancers as a whole. Perhaps pathologists are currently more conservative in diagnosing these tumors as malignant. Conversely, adenoid cystic carcinomas of the salivary gland increased in relative frequency from 13 to 18%.

In addition to data for 1973-77, data for 1978 and 1979 are also available. Since New Orleans data are not available past 1977, comparison of numbers of cases and age-adjusted rates for 1978 and 1979 with those for 1973-1977 must be interpreted in that light. Currently, data exist on 70,082 and 70,327 malignant cases for 1978 and 1979, respectively, for the nine geographic areas excluding Puerto Rico and 4,980 and 5,034 malignant cases for 1978 and 1979, respectively, from Puerto Rico.

Because of difficulties in estimating the population of the various minority and ethnic groups, incidence rates for only whites and blacks have been produced for 1978 and 1979. The rates (age-adjusted to the 1970 U.S. population) for all cancer sites combined for the nine geographic areas combined are as follows:

	White Males	Black Males	White Females	Black Females
1978	386.8	470.1	302.0	295.4
1979	380.8	482.7	295.3	305.8

Thus, in 1979 for the first time since cancer incidence data have been available (1935), black females had higher rates than white females. However, of the nine geographic areas only three (Atlanta, Detroit and San Francisco) have significant black populations. The 1979 age-adjusted incidence rates for females for these areas were as follows:

	White Females	Black Females
Atlanta	308.7	267.1
Detroit	290.6	314.3
San Francisco	320.3	297.3

Thus, the only one of the three areas in which black females had higher incidence rates than whites was Detroit. It should be noted that Detroit has brought suit against the Bureau of Census to correct for the underenumeration of blacks in the population. Therefore, if the suit is successful and black populations are adjusted, the higher rate among blacks may disappear entirely. This example emphasizes the importance of having accurate denominator (population) data in comparing incidence and mortality rates among the various geographic areas and race/ethnic groups. Hence, it seems clear that NCI should not publish incidence and mortality data beyond 1977 until results of the 1980 census have become fully available to the public.

With respect to survival data, tapes will be submitted to NCI in September 1981 containing follow-up on all patients diagnosed 1973-79 and followed at least through December 1980. This will allow the calculation of five-year survival rates for patients diagnosed 1973-75, three-year survival rates for patients diagnosed 1973-77 and one-year survival rates for patients diagnosed 1973-79. If the data are sufficiently improved over the previous submission, i.e., the percent lost to follow-up decreases, it is anticipated that a survival publication comparing the survival experience of patients in the various geographic areas and among the various race/ethnic groups can be prepared. Based on current data, survival experience among the various areas varies considerably. However, this is felt to be an artifact since those areas with the poorest survival rates are the areas with the highest percentage of patients lost to follow-up. If the assumption is made that all patients lost to follow-up are actually still alive, the survival experience of the various areas becomes more similar, but the resulting survival rates are overestimates of the true survival experience of cancer patients.

Significance to Biomedical Ressearch and the Program of the Institute:

Continuing information on both cancer incidence and patient survival is essential so that the nature and magnitude of the cancer problem can be determined and changes over time can be assessed. As an important step toward prevention, continuing analysis of variations in cancer incidence across subgroups of the population can lead to specific etiologic hypotheses for further testing. This in turn may lead to the identification of risk factors which can be brought under control. Through continuing analyses of survival data, the Program can provide important clues to improved treatment methods. The maintenance of population-based cancer registries provides a data base against which some of the major programs of the Institute can be assessed, for example, the impact of specific cancer control programs can be measured to determine the extent to which these programs are meeting their stated goals. Data from cancer centers can be compared with the population-based data to determine in what ways cancer center patients and the treatment they receive differs from that of the general population.

Since the SEER Program identifies all cases of cancer diagnosed in defined populations, it is a valuable resource for case-control studies to identify possible etiologic agents. A major advantage is that case-finding techniques are already in place, and it is possible to modify them to permit very early identification of the cancers of interest so that patients, rather than proxies for the patients, can be interviewed. Furthermore, population controls can be used, thus avoiding a number of problems posed by the use of non-cancer hospital controls. Two major studies of this type have been carried out in most of the SEER locations. One was aimed at studying the relationship of bladder cancer and the use of artificial sweeteners and the other was attempting to assess the impact of ozone depletion on the occurrence of non-melanoma skin cancer. The use of the SEER mechanism to study the relationship between the use of oral contraceptives and cancers of the breast, ovary and endometrium is now underway.

The Program serves as the major data base for cancer incidence and mortality data in the United States. Data are given to other government agencies, the American Cancer Society, the World Health Organization, the International Agency for Cancer Research, Congressional Offices and the general public. In addition to those requests received by the NCI Office of Cancer Communication, the Demographic Analysis Section received 123 telephone requests for data during the period October 1, 1980 through February 28, 1981 for an average of 7 telephone requests for data per week. In general, these requests were from the news media and the general public and could be answered with data already available. However, one request from another government agency took 23 man hours of time to prepare the appropriate response. Without the SEER data base, it would be impossible to respond to these telephone inquiries. It is estimated that 15% of professional staff time is devoted to responding to either verbal or written requests for data.

Proposed Course:

1. The Program is currently being reviewed by an ad hoc group which is a subcommittee of the Board of Scientific Counselors of the Division of Cancer Cause and Prevention. This group will make recommendations regarding the size and scope of the program. Until this group makes its report, the future of the program cannot be stated with absolute accuracy. It is anticipated, however, that the program will continue and will be expanded in two ways. First of all, the data base will be expanded to include three new data items: occupation/industry in which the patient is/was employed, type of cancer surgery performed, and for patients with melanoma of the skin, exact anatomic location of the lesion (e.g., finger, palm, wrist, lower arm, etc. vs. "upper extremity"). Second, the data base will be expanded to replace coverage lost by the discontinuation of New Orleans in the program and to augment the coverage of the black and Hispanic populations represented in the program. Further consideration may again be given to relaxing the requirement of active follow-up of all cancer patients by each program participant.

2. NCI will continue to work with several program participants in upgrading and maintaining a data management system. Any new program participant without an ongoing operational data processing system already in place will be required to use the data management system currently being installed in the Connecticut Registry.

3. A publication detailing the cancer patient survival experience of patients diagnosed 1973-79 will be prepared. If sufficient census data become available, incidence and mortality data for 1978-1980 will be prepared.

4. To assist in the tumor registry training program and to provide guidance to SEER Program participants and others concerned with cancer registration, a number of publications have been developed and distributed. These include manuals that set out clear guidelines for collecting and coding the data to be submitted to NCI, as well as books that can be used for on-the-job training of personnel responsible for carrying out the functions required for data extraction and coding. The demand for these publications has been large and mailings have been made to a variety of medical personnel and facilities. A new book documenting antineoplastic drugs is ready for distribution. Additional books on statistical and epidemiological methodology and computer assistance for the tumor registrar are being developed. Earlier books on anatomy and extent of disease are being revised.

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6) Percy, C., Stanek, E., and Gloeckler, L.: Accuracy of cancer death certificates and its effect on cancer mortality statistics. Am. J. Public Health 71: 242-250, 1981.

7) Shambaugh, E.M. (Ed.): Self Instruction Manual for Tumor Registrars, Book Four: Human Anatomy as Related to Tumor Formation. U.S. Department of Health and Human Services, Publication No. (NIH) 80-2161. Washington, D.C., U.S. Government Printing Office, 1980, 221 pp.

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11) Young, J.L., Jr., Percy, C.P, Asire, A.J., Berg, J.W., Cusano, M.M., Gloeckler, L.A., Horm, J.W., Lourie, W.I., Pollack, E.S., and Shambaugh, E.M.: Cancer Incidence and Mortality in the United States, 1973-77. In Young, J.L., Jr., Percy, C.L., and Asire, A.J. (Eds.): National Cancer Institute Monograph 57: Surveillance, Epidemiology and End Results Program: Incidence and Mortality Data, 1973-77. Department of Health and Human Services, Publication No. (NIH) 81-2330. Washington, D.C., U.S. Government Printing Office, 1981, pp. 1-9.

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October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Studies of Therapeutic End Results and Prognostic Factors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M.H. Myers, Chief,	Biometric Research & Analytic Studies Section,	BB NCI
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COOPERATING UNITS (if any)

Health Insurance Plan of Greater New York

LAB/BRANCH
Biometry Branch, Field Studies and Statistics Program

SECTION
Biometric Research and Analytic Studies Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The survival advantage of white over black patients with cancer of the urinary bladder and uterine corpus was due in part to a more favorable stage distribution for white patients. However, black/white survival differences were observed for patients with localized disease. Study of patients with breast cancer revealed higher than expected numbers of subsequent cancers of the second breast, colon, rectum, lung, corpus, ovary, buccal cavity and melanoma. From 1969-71 to 1973-79 incidence rates for malignant mesothelioma among white males increased from 5.5 to 9.3 per million population. For white females, the trend was from 2.1 to 2.7 per million. Truck drivers were found to be at elevated risk of developing bladder cancer. Risk increased with increased duration of truck driving and for drivers of trucks with diesel engines. A study of uranium miners indicated that the probability of malignant transformation increased in proportion to degree of cellular atypia and that there is a possible synergistic effect of smoking and radiation exposure on transition to more severe cytologic states. Survival rates for patients with metastatic non-seminomatous testicular cancer improved substantially from the early to the late 1970's. This observation is in parallel to introduction of developed combination chemotherapy protocols.

Project Description

Objectives:

To conduct research in cancer etiology, screening for early detection, prognosis and in statistical methodology.

To provide consultation to other divisions of NCI, to other institutes of NIH and to non-governmental groups on collection and analysis of data on human populations, on design and analysis of special studies.

Methods Employed:

A wide variety of statistical procedures and principals are used for designing studies and analyzing results. Some of these are standard techniques, while others are developed for handling specific conditions dictated by the subject matter.

Major Findings:

1. Recently published survival results have indicated that, in general, white cancer patients had a more favorable survival experience than blacks. Part of the white survival advantage was explained by a more favorable stage distribution at diagnosis: black patients presented more frequently than whites with metastatic disease. Urinary bladder for both men and women and uterine corpus for women are sites which exhibited the largest black/white survival differences which persisted after adjustment for stage and age.

2. Work has continued on the study of multiple primary cancers utilizing the historical data from the Connecticut Tumor Registry (CTR). The focus over the past year has been on the development of computer software which will facilitate multiple primary studies utilizing both the historical data from Connecticut and the more recent data from all the SEER participants. Such studies may aid in the quantification of the effects of certain treatments (radiotherapy, chemotherapies, etc.) on the subsequent risk of various tumors. These studies should also permit assessment of the effects of age and stage of disease of a first tumor on any subsequent cancer experience.

Multiple primary studies are currently in progress utilizing this resource and are being done with varying degrees of collaboration with members of the Environmental Epidemiology Branch. The subsequent cancer experience of cervical cancer patients has been studied. Some of the findings included excess risks of second leukemias and cancers of the lung, bladder, kidney, and genital organs for those patients treated with radiation. The subsequent cancer experience of breast cancer patients is currently being studied. Second breast tumors were found to occur in substantial excess in patients <45 years old during the first 10 years after diagnosis. The presence of regional lymph node involvement was also found to be associated with increased excess risk of second breast tumors in the <45 year age group; the risk was elevated but smaller for those 45 and over. Cancers of other sites occurred more frequently than expected after a

primary breast cancer: colon, rectum, lung, corpus, ovary, buccal cavity, and melanoma. The excess risk for these sites was particularly elevated for breast cancer patients <50 years old at diagnosis. The excess risk of subsequent cancer of the corpus was elevated for patients 50+ years old. There was some indication of an association between the use of adjunctive radiation for the primary breast cancer and elevated risk 10+ years after diagnosis of second breast and lung tumors.

3. One hundred ninety-one cases of in situ breast cancer were studied with regard to the subse- quent development of contralateral breast cancer (subsequent cancers of the other sites were rare). The patients, drawn from 508 cases diagnosed 1955-71, were reported by the California and Connecticut Tumor Registries. Slides for 295 patients were available and reviewed by two NCI pathologists; 201 had an unequivocal diagnosis of unilateral in situ carcinoma (10 were excluded since they had bilateral mastectomies). Only women with lobular carcinoma in situ had a statistically significant increase in subsequent contralateral carcinoma (Observed to expected ratio of 6.6).

4. Data from the Third National Cancer Survey (TNCS) (1969-1971) and from the SEER Program (1973-1979) indicated that for white males, incidence rates for malignant mesothelioma increased substantially from 5.5 per million (1969-1971) to 9.3 per million. The trend for white females over the same time period was 2.1 to 2.7. Residents of Seattle and San Francisco-Oakland had significantly higher rates than residents of other areas included in the SEER Program.

5. The relationship between occupation and cancer of the lower urinary tract was examined in Detroit by means of a population-based case-control study conducted as part of the National Bladder Cancer Study. Three hundred three white male patients with transitional or squamous cell carcinoma of the lower urinary tract and 296 white male controls, selected from the general population of the study area, were interviewed to obtain lifetime occupational histories. The findings suggested that truck drivers have a significantly increased risk of lower-urinary-tract cancer (relative risk = 2.1; 95 percent confidence interval = 1.4 - 4.4). A significant trend in risk with increasing duration of employment as a truck driver was apparent ($p = 0.004$); the relative risk estimated for truck drivers employed at least 10 years was 5.7. Truck drivers with a history of operating vehicles with diesel engines experienced a significantly elevated risk (relative risk = 11.9, 2.3 - 61.1), but diesel exposure did not completely explain the increased risk observed among truck drivers. Nonsignificant excess risks were also seen for tool & die makers, as well as for workers in several other industries and occupations. Employment in the motor vehicles manufacturing industry was associated with little or no excess risk of lower-urinary-tract cancer (relative risk = 1.1; 0.8 - 1.5).

6. Findings to date in the Health Insurance Plan Study strongly suggest the usefulness of the annual screening for breast cancer using clinical examination plus mammography. Over a thirteen year period of follow-up, the study group has about one-third less mortality from breast cancer than the control group. This reduction appears to be concentrated among women 50 years of age and older. The differential between study and control cases in fatality from breast cancer is

due almost entirely to exceptionally better survival among cases detected through screening. Both the clinical examination and mammography contributed to this favorable situation, but the magnitude of the independent contribution of each modality is difficult to determine.

7. A study of lung cancer in uranium miners was continued in collaboration with Dr. Thomas Mason of NCI, Dr. Geno Saccomanno of Grand Junction, Colorado and Dr. Victor Archer of Salt Lake City, Utah. The natural history of lung cancer as defined by sputum cytology and the influence of smoking, radiation and demographic variables on etiology are being investigated using a descriptive epidemiologic approach and stochastic modeling concepts. It was found that the probability of malignant transformation increases with increasing severity of cellular atypia; however, for intermediate stages of atypia, there was also a significant probability of disease regression. An expanded analysis examining transition probabilities and sojourn times for cytologic states has revealed a possible synergistic relationship between smoking and radiation exposure with regard to transitions toward more severe cytologic states. The results of this project have implications for lung cancer prevention programs and industrial safety regulation legislation.

8. Data from the Third National Cancer Survey were used to investigate the associations of lung and bladder cancer incidences with income and education, as well as to determine the extent to which racial differences in socioeconomic (SE) distribution account for the observed racial differences in rates. Median family income and median years of education by census tract were used to define the SE groups. The relative risk (RR) for lung cancer showed strong significant inverse associations with both income and education among both white and black males, and the effect of income exceeded that of education. The black/white RR, significantly >1.0 before SE adjustment, was reduced to nonsignificance with adjustment for education and became <1.0 with adjustment for income. Bladder cancer, in contrast, showed a significant positive association with education and nonsignificant positive trend with income among white males. Although non-significant, a positive association with education was also suggested among black males. SE adjustment reduced the racial differences in rates, but the excess risk among white males remained large. Strong trends with either SE variable were not observed for lung or bladder cancer among females of either race.

9. An increase in the reported incidence of endometrioid and clear cell cancer of the ovary occurred in the United States during the 1970's, while no change occurred in the overall incidence of ovarian cancer. The authors cannot rule out that this was due to a shift in the criteria for histologic classification or improved coding, although these seem unlikely to account entirely for the change. In the four areas where the trend for endometrioid and clear cell cancer of the ovary was examined, the proportional increases in its occurrence were correlated with the proportional increases in the occurrence of carcinoma of the uterine corpus. The concomitant trends and the biologic similarities between these histologic types of ovarian cancer and the uterine cancers suggest that common etiologic factors may be involved. The role of postmenopausal estrogen use in the etiology of ovarian cancer must be clarified by further epidemiologic studies, but such studies should take tumor histology into consideration.

10. The demographic characteristics of pancreatic cancer have been studied based on data from the TNCS and the SEER Program. Incidence and mortality rates have continued to increase over time but at a much slower pace than in earlier years. Income and education levels had little influence on incidence rates for this disease among either black or whites. Only small and statistically insignificant differences in pancreatic cancer incidence rates among urban and rural residents were found in the data analyzed. The predominant demographic characteristics are the excess risks among men compared to women and among blacks compared with whites. These demographic patterns suggest that no single factor previously associated with pancreatic cancer, such as cigarette smoking, plays a predominant role in the etiology of this disease. Other work in this disease by the Pancreatic Task Force of the American Joint Committee has led to a TNM classification. A surgical evaluative staging scheme was developed based on the analysis of data collected on 924 cases of cancer of the exocrine pancreas seen at 14 clinics and hospitals.

11. An analysis is being made of the cancer experience of the elderly, those 65 years of age and older, a group which comprises 11 percent of the population and experiences one-half of all new diagnoses of cancer and 60 percent of the deaths from cancer. Mortality data for the United States indicate that lung and bronchus cancer leads as a cause of cancer death among males 65 and older, whether white or black, with prostatic cancer and colon cancer next in frequency. Among females, white or black, 65 and older, breast cancer and colon cancer deaths lead with equal relative frequency and lung and bronchus cancer deaths follow. Before age 75, breast cancer deaths exceed those from colon cancer, but from age 75 on, colon cancer is more important as a cause of death, for both white and black females.

12. Collaboration with investigators from the Soviet Union on survival of breast cancer patients is awaiting submission of approximately 2,000 USSR cases. Data for the U.S. part of the study has been assembled and further acquisition of follow-up information is continuing. Preliminary analyses indicate more frequent use of chemotherapy as an adjunct to surgery for Soviet patients (sample Soviet data). Several more years must pass before 5-year follow-up will be possible.

13. Relative survival rate methodology was used to investigate the relationship between cancer patient survival and mortality attributed to the diagnosed cancer. This was done for breast cancer, lung cancer, colorectal cancer, and cancer of the prostate utilizing data from the Connecticut Tumor Registry for the calendar period 1949-73. For each site, except prostate, it was found that relative survival estimates of deaths were consistently higher (on the order of 10 to 20 percent) for each calendar year relative to the corresponding number of deaths based on death certificates. For cancer of the prostate, relative survival methodology gave even higher estimates of the number of deaths attributable to that cancer than death certificate information. The analysis seems to indicate that cancer patients are at increased risk of dying of causes other than cancer.

14. The dramatic improvements in the survival experience for white children diagnosed with acute leukemia were analyzed using data collected through hospitals participating in the National Cancer Institute's End Results Group Program between 1950 and 1973. Children under 15 years of age diagnosed with both acute lymphocytic leukemia (ALL) and acute nonlymphocytic leukemia (ANLL) showed moderate improvements in the 1950's, but beginning in the 1950's those with ALL did far better, with statistically significant differences at the 0.05 level noted between their 3-year survival rates for all cohorts analyzed between 1960 and 1973. For the 1970-1973 cohort, 3-year survival rates were 49 percent and 20 percent for ALL and ANLL, respectively, and 5-year survival rates were 34 percent and 12 percent. Between 1950 and 1976 the age-adjusted incidence rate for all childhood leukemias remained relatively stable in a sample of five geographic areas, changing from 4.6 per 100,000 children under 15 years of age to 4.3 per 100,000. In contrast, the corresponding age-adjusted mortality rate fell approximately 45 percent over the same period, from 4.4 per 100,000 to 2.4 per 100,000.

15. Incidence, mortality and survival rates for testicular cancer have been studied. Disease incidence rates have increased only moderately from 3.2 per 100,000 in 1973 to 3.8 per 100,000 in 1979 (SEER data). U.S. mortality rates decreased only moderately from 0.8 per 100,000 in 1973 to 0.7 per 100,000 in 1977, but then fell precipitously to 0.5 in 1978 (the most recent year for which national data were available). When survival rates for SEER cases diagnosed during the years 1973-76 and 1977-79 were compared, no change in prognosis over time was seen for seminomas of the testes. However, prognosis improved significantly for nonseminomas, particularly for those with disseminated disease. Improved survival of nonseminomatous cancers of the testes can be attributed to the development of new combination chemotherapy protocols for treatment of these malignancies. The recent decline in national mortality rates suggests that knowledge of this advance in cancer therapy has been effectively disseminated to practitioners in the U.S.

16. A review of the data for all female breast cancer patients <36 years old diagnosed in the Province of Saskatchewan, Canada, during 1945-71 has been completed and some preliminary analyses of the data have been done. Five-year survival of nulliparous patients was significantly better than that for parous patients (0.83 versus 0.63). Further, parous patients' survival appeared to be related to the number of children: the five-year rates for parous patients with one child, two children, and three plus children was 0.76, 0.66, and 0.58, respectively.

17. Further analysis of the selected series of soft tissue sarcoma cases revealed that some cases previously considered unstageable because of unknown tumor size can be staged because of known information regarding status of regional lymph nodes and grade. Two other analyses are being carried out: 1) study of recurrence and 2) a multivariate analysis which will assess the relative weight of the following factors with respect to prognosis: grade, location of tumor, symptoms, X-ray findings; tumor size and direct extension.

18. An analysis of prognostic factors in thyroid cancer indicated that prognosis is unusually good even for patients with distant metastases. We are currently investigating joint prognostic significance of extension of tumor, histologic type, regional lymph node involvement, distant metastases, and age. Age (<45, 45+) seems to be of major prognostic importance (much poorer survival for patients 45 or older).

19. A staging scheme was devised for stomach cancer using 2937 SEER patients diagnosed 1977-1978 with survival recorded through 1979. The American Joint Committee (AJC) Classifications (1978) were used to summarized the more detailed SEER extent of disease data. An evaluation of the scheme was made using survival analysis techniques. The study concluded that the detailed SEER extent of disease information was compatible with the AJC system and served to further refine stage subcategories. A similar analysis of SEER colon cancer data demonstrated the compatability of the detailed extent of disease information with the AJC classification. Preliminary analysis has already resolved a difference of opinion between the AJC and the International Union Against Cancer Staging Committee regarding the relative prognosis for patients with involvement of organs beyond the colon.

20. A review of available autopsy slides for Hodgkin's disease patients treated at the Clinical Center through 1969 has been completed by Dr. Thomas Grogan (now at the University of Arizona). There were 215 Hodgkin's disease deaths during the period of the study, 120 of which were included in this study. The 120 deaths appeared reasonably representative of all the deaths. A not too unexpected finding was that deaths which occurred among patients diagnosed and treated during 1965-69 were more likely treatment-related than those deaths among patients diagnosed and treated prior to 1965. Attempts are being made to further characterize sites of involvement at death by calendar period and histologic type and assess changes (progression) in histologic type between diagnosis and death.

21. Work has continued on the development of theory for the evaluation of screening programs for the early detection of disease using methodology from renewal theory. Effort has focused on the theory of repetitive screening in the context of an evaluation trial. Topics being investigated include age-dependence, parameter estimation, lead time, length-biased sampling and mortality measures. A simulation study was begun to investigate the impact of screening frequency and disease natural history on lead time variables.

22. An age-dependent mathematical model of screening is under development. The basic variables are age at entry into the preclinical state, preclinical state sojourn time, and age at screening or observation. The three variables are allowed to be dependent, with the age at screening or observation having an arbitrary distribution over a bounded range. The joint density functions of the three variables for individuals who are detected by screening or whose disease surfaces clinically between screening tests have been derived. These results lead to a more complete understanding of length bias in screening than has been possible previously. A numerical investigation of length bias at a prevalence screen as a function of screening parameters was initiated. In addition,

concepts from the model have been applied to the development of a new technique for lead time estimation. Estimation of the lead time distribution in repeated screening was investigated.

23. In a case-control study conducted in Detroit as part of the National Bladder Cancer Study, the relationship between artificial sweetener consumption and lower-urinary-tract cancer was evaluated using both a hospital control group and a population control group. To obtain histories of artificial sweetener use, an interview was administered to 391 patients with transitional or squamous cell carcinoma of the lower-urinary tract, 305 hospital controls, and 440 population controls. We observed a higher proportion of artificial sweetener users among hospital controls than among population controls. Relative risks estimated with hospital controls were consistently lower than those estimated with population controls. For males, when controls with conditions associated with artificial sweetener use were excluded from the hospital control group, relative risks were similar to those estimated with population controls. For females, little or no change in relative risk was observed after exclusion of hospital controls with conditions associated with artificial sweetener use, but these estimates were based on small numbers and tended to be unstable. This study indicated that, in hospital-based case-control studies of the effects of artificial sweeteners, controls with conditions related to artificial sweetener use should be excluded from analysis to obtain unbiased estimates of relative risk.

Significance to Biomedical Research and the Program of the Institute:

Many of the benefits of the consultation program are long range in nature and consist of setting up record systems and study situations which enlarge the potential study resources of NCI and related organizations. The research objectives of the Institute are promoted by continued work on the integration of various study techniques, e.g., retrospective and prospective studies, analyses of mortality data and morbidity surveys, epidemiologic investigations and cancer registries. An example of the interaction between epidemiologic and end results investigations are studies of prognostic factors and the role of these factors in cancer etiology.

Proposed Course:

The emphasis of this project has been on examining patterns of cancer patient survival in relation to therapy, extent of disease, histologic type, etc. This kind of activity will continue as new studies of factors related to patient survival are conducted. Effort will be devoted to developing a better understanding of the differences in survival between white and black patients. Initial studies will focus on cancers of the bladder and uterine corpus, sites for which the racial differences are largest.

Examination of Connecticut data for excess risk of subsequent primary cancers will extend to sites other than breast cancer. The same analytic approach will be applied to data from the SEER Program so that risks of second tumors as a function of age, stage, histologic type and treatment of the primary cancer can be assessed.

Appropriate data resources will be compiled for assessing long-term (30+ year) trends in cancer incidence. Analyses will focus on trends in age-adjusted and age specific rates by sex and race.

Analytic studies for investigative factors related to cancer etiology will be developed. Special emphasis will be placed on environmental or occupational determinants that can lead to preventive measures.

Activities will continue on the development of methodology for the design and analysis of screening programs. New models for the analysis and comparison of screening designs and measures of effectiveness will be developed. The role of lead time and length-biased sampling in the analysis and interpretation of screening data will be studied. It is anticipated that the HIP breast cancer of screening study, a contract of the Biometry Branch, will continue through at least 15 years of follow-up of the entire study population, and that the results of modeling research will be applicable to the HIP data as well as to data from screening programs for other cancers.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04258-13 B

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Studies of Cancer Incidence and Mortality and Related Etiologic Factors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I. :	Earl S. Pollack, Chief	BB NCI
	Haitung King, Senior Research Scientist	BB NCI
	Joseph Scotto, Health Services Director	BB NCI
Others:	James L. Murray, Contract Prgm. Mgmt. Ofcr.	BB NCI
	Frances Locke, Statistician	BB NCI
	Tu Ji-Tao, Visiting Fellow	BB NCI

COOPERATING UNITS (if any)
University of Bergen, Norway; Kuakini Hospital, Honolulu; University of Minnesota; Universidad del Valle, Cali, Colombia; Louisiana State University; Shanghai Cancer Registry

LAB/BRANCH
Biometry Branch, Field Studies and Statistics Program

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Research on cancer incidence and mortality continued in a number of studies on specific cancers. Migrant populations from Japan, Norway and China are being studied in an attempt to identify factors associated with the occurrence of specific cancers, with particular emphasis on diet in relation to gastrointestinal cancers. Among the Hawaiian Japanese, an inverse relationship was found between serum cholesterol level and the subsequent occurrence of colon cancers, with the risk of colon cancer highest among those with low initial serum cholesterol levels. Among Norwegian migrants to the U.S. an increased risk of colon cancer was found among those with heavy consumption of fresh or frozen fish and smoked or salted ham or pork. There were also suggestions of increased risk of colon cancer among those with high beer consumption. Among the Chinese in Shanghai, the incidence of cancer of the esophagus, stomach, liver, and cervix is exceptionally high compared to that among the San Francisco Chinese and the incidence of colon, breast and prostate cancer is very low. These comparisons are being explored further.

Project Description

Objectives:

To describe and analyze cancer morbidity and mortality in human populations; to identify factors that may be associated with the occurrence of cancer; and to identify causative or promoting factors for specific sites of cancer through comparison of high-risk and low-risk populations, taking advantage of natural experiments such as comparison of migrant groups with their country of origin.

Methods Employed:

Several projects are reported on here, all of them primarily descriptive epidemiological studies. The Japan-Hawaii Cancer Study is a prospective study in which an initial physical examination was given and a detailed questionnaire administered through interview with primary emphasis on dietary information on a cohort of 8,006 Japanese men in Hawaii. The cohort is then followed through hospital records, death certificates and other sources to identify cases of specific cancers that have occurred since the initial contact. A similar methodology has been employed in the Luthern Brotherhood Study in Minnesota on a cohort of Norwegian migrants to the United States but the initial information was obtained through interview with no physical examination being given; the same is true with the cohort of individuals being studied in Norway. The analysis of data on Chinese migrants has been carried out through comparisons of mortality data between Taiwan, Singapore, Hong Kong, and more recently the Peoples Republic of China, and Chinese populations in the United States. Comparisons of cancer incidence data are now being made through the use of the Shanghai Cancer Registry and the cancer registries in San Francisco and Hawaii.

Major Findings:

Japan-Hawaii Cancer Study:
A cohort of 8,006 Japanese men, born between 1900 and 1919 and living in Hawaii, has been followed since the mid-1960s to relate dietary factors, as well as a host of demographic and physical examination variables, to the occurrence of cancers of the stomach, colon, rectum, lung and prostate. Until recently the number of cases of each cancer has been too small for detailed analysis. During the past year analysis of the probability of occurrence of specific cancers in relation to serum cholesterol levels determined at examination revealed that there is a strong suggestion of a negative relationship between serum cholesterol and colon cancer. This relationship was not apparent for the other cancers. The incidence of colon cancer was high among those with serum cholesterol under 180mg/100ml. Similar findings have emerged from other prospective studies, notably the Framingham and Hiroshima-Nagasaki studies. The National Heart, Lung and Blood Institute is sufficiently concerned about this issue that they have assembled investigators, representing 16 prospective studies that have examined the relationship between serum cholesterol and cancer, to participate in a workshop to explore this relationship further.

Since there are now approximately 100 colon cancer cases and 67 rectal cancers, the possible relationship between alcohol consumption and these cancers will be explored. A detailed recoding of the 24-hour diet recall questionnaire is being carried out for colon cancer cases and two matched controls drawn from the cohort. The purpose is to attempt to measure intake of dietary fiber, vitamin A, vitamin C, and other nutrients to test the hypothesis that these nutrients have a protective effect. As a corollary to this a pilot study is being carried out using serum from the initial examination to assay for vitamin A and vitamin E levels. If the results of the pilot are favorable, a study will be carried out on the association between vitamins A and E and specific cancers in the study.

Work is continuing on the characterization of mutagens occurring within the stomach and colon. Two new mutagens have recently been identified -- one in the central mucosa of the stomach and the other in the mucosa of the sigmoid colon. Since these mutagenic substances cannot be related to any drug received by the patients during the course of their treatment, it is possible that these substances are from dietary or environmental sources.

Norwegian Migrant Studies:
The detailed analysis of the Norwegian data is now being carried out and results are not yet available. Preliminary analysis of the Luthern Brotherhood data reveal the following:

- There is an increased risk for colon cancer among persons with large consumption of fresh/frozen fish, smoked/salted ham or pork.
- The risk for colon cancer is enhanced for heavy users of meats/fats only when there is correspondingly low use of vegetables or grain fiber.
- Beer consumption appears to be positively associated with colon cancer mortality.
- There were no significant associations between alcohol consumption and mortality from cancers of the rectum, stomach, lung, bladder, prostate or leukemia.
- Positive associations were found between stomach cancer mortality and consumption of cooked cereals.
- Milk consumption was positively associated with stomach cancer mortality.
- There was no clear relationship between vitamin C index and stomach cancer mortality.
- Consumption of total meat, beef and fresh pork ham was not related to stomach cancer.
- There was a suggestive positive association between stomach cancer mortality and consumption of chicken, bacon/side pork, smoked/salted ham or pork, salted fish and total fish.
- There was a negative association between cancer of the pancreas and total meat consumption.
- Cancer of the pancreas mortality was positively associated with alcohol consumption, when controlling for the effects of age and cigarette smoking.
- Vitamins A and C may be protective against lung cancer, and perhaps stomach cancer and rectal cancer. The Minnesota study shows a possible increased risk between dietary vitamin A and colon cancer or pancreas cancer. These findings are highly tentative at the present time.

Chinese Migrant Study:
Work was continued on a study of health indicators in China in comparison with the experience of Chinese migrants to the U.S., covering general mortality, cancer, heart disease, nutrition, mental health, occupational health, etc. Comparisons have been made using mortality experience for the U.S.-Chinese and those of Chinese in Taiwan, Singapore and Hong Kong. More recently as a result of the 1973-75 first national mortality survey by cause in China it is possible to compare U.S.-Chinese cancer mortality rates directly with those for the Peoples Republic of China. Preliminary analysis indicates that overall cancer mortality risk is similar for females in both countries but for the U.S.-Chinese males, rates were higher among the foreign born than among those in China and lower among native born. However, for specific sites, large differences have been noted. Sharp reductions in mortality risk among migrants were shown for stomach, esophagus, and liver cancer whereas increased rates were noted for lung, and colon cancer. Among female migrants changes in risk from the country of origin to the United States followed a similar pattern for these sites but the rates were low for both the home and host populations. Of particular interest was the sharp downward transition for cervical cancer and an increase in the risk for breast cancer among migrants.

Comparison of cancer incidence between Shanghai and the United States:
Doctor Tu Ji-Tao, an epidemiologist with the Shanghai Cancer Institute, arrived at the end of February, 1981 to spend a year in the Biometry Branch as a Visiting Fellow. He brought with him cancer incidence and mortality data covering the period 1972 through 1979. One of his initial primary interests was to compare the Shanghai incidence rates for specific cancers with those for some of the Chinese populations in the United States. In the preliminary analysis of these data, the San Francisco Chinese and the Hawaiian Chinese populations were used for comparison as well as the white population for the entire SEER Program. As might be expected, some very striking differences emerged. Some of the major differences are as follows: The incidence rate for esophageal cancer was about four times the rate in each of the two Chinese populations in the U.S., and the same is true for stomach cancer. For colon cancer, on the other hand, the Shanghai rate is about one-quarter that of the U.S.-Chinese rates. The liver cancer rate in Shanghai is about twice that for the San Francisco Chinese, four times the rate for the Hawaiian Chinese and about 15 times the rate for the U.S. whites. The Shanghai prostate cancer rate, on the other hand, is less than one-tenth the rate in the Chinese populations in the U.S. and less than one-twentieth of the rate among U.S. whites. The rate for female breast cancer in Shanghai was about one-third that in the U.S.-Chinese populations. The cervical cancer rate was about double to triple the U.S.-Chinese rates, while the rate for cancer of the uterine corpus was about one-sixth the rate for the San Francisco Chinese and about one-tenth the rate for the Hawaiian Chinese. The patterns of these rates will be studied in more detail in order to develop specific hypotheses for further study both in Shanghai and in the Chinese populations in the United States.

Some preliminary investigation has been made of the migrant status of both the San Franciscan and Hawaiian Chinese populations. It is estimated that approximately 80 percent of the San Francisco Chinese population originated from the Canton area and a much larger proportion of this population are

relatively recent migrants compared with the Chinese population in Hawaii. Since there does not appear to be any substantial group in the United States that originated in Shanghai, it is not possible to carry out a more traditional migrant study as has been done, for example, among the Japanese population. One of the reasons for this is that, based on mortality data in China, there are vast differences in rates among various areas of China. For example, mortality rates for cancer of the nasopharynx in the Canton area are about five times those in Shanghai. The comparison of the Shanghai incidence data for cancer of the nasopharynx with those of the San Francisco Chinese show the Shanghai rate to be only about one-third that of the San Francisco Chinese and even slightly lower than that of the Hawaiian Chinese. On the other hand, the mortality rate for cancer of the esophagus in Shanghai is more than three times that of the Canton area and for cancer of the stomach about five times that of the Canton area. Thus, some mortality studies are also being planned comparing mortality for various populations in the U.S. with mortality rates in specific areas of China to begin to understand possible reasons for some of these differences.

Epidemiology and Geographic Pathology of Cancer in South American Populations: This project has been carried out on contract from the Biometry Branch since January 1975. In view of the fact that no staff member of the Biometry Branch has a direct involvement in the project, the research team was encouraged to apply for grant support to continue to carry out this research. A grant was awarded in April 1981 and the work will continue under that mechanism.

Significance to Biomedical Research and the Program of the Institute:

These studies attempt to identify high and low risk population groups which can then be studied further for the identification of possible etiologic agents. These descriptive studies permit the development of more specific hypotheses for analytic studies. The study of migrant populations attempt to suggest environmental factors that may be associated with the incidence of certain forms of cancer. The assumption is that if these factors can be identified it may then be possible to initiate preventive measures thus reducing the risk from those particular forms of cancer.

Proposed Course:

Further analysis of the Hawaiian Japanese data will be carried out, particularly on the relationship between diet, and some of the dietary components, and the occurrence of cancer of the colon and rectum. Systematic analysis of the data from the Norwegian studies will continue. The analysis of cancer incidence in Shanghai compared with that in the San Francisco Chinese and the Hawaiian Chinese will be carried out in depth with a view toward formulating specific studies to identify factors associated with the large differences between these populations; and exploratory study of cancer incidence and mortality among Puerto Ricans in New York City will be carried out. If successful, this will lead to some comparative studies between that population and Puerto Rico, where a SEER registry is now located.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04260-21 B

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Consultation on Clinical Trials

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. P. Byar	Chief, Clin. & Diag. Trials Section	BB NCI
OTHER:	M. H. Gail	Medical Statistical Investigator	BB NCI
	S. B. Green	Medical Researcher	BB NCI
	D. L. Levin	Senior Investigator	BB NCI
	L. R. Muenz	Mathematical Statistician	BB NCI
	D. K. Corle	Computer Systems Analyst	BB NCI
	L. V. Rubinstein	Staff Fellow	BB NCI

COOPERATING UNITS (if any)

Division of Cancer Treatment, NCI
Division of Resources, Centers, and Community Activities, NCI

LAB/BRANCH
Biometry Branch, Field Studies & Statistics Program

SECTION
Clinical & Diagnostic Trials Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.5	3.5	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to provide consultative services in statistical and epidemiological methodology in the design, interpretation, and evaluation of clinical trials and other studies requiring this kind of expertise. For some trials the section provides full statistical support including development of detailed study plans, randomization of patients, collection, processing, editing, and analysis of data, and preparation of scientific papers. At present, members of the Section are involved in studies concerned with prostate cancer, bladder cancer, testis cancer, breast cancer, lung cancer, brain tumors, and mycosis fungoides. In addition to these major projects, a variety of other consultative activities, including review of scientific articles, arise in response to special requests.

PHS-6040
(Rev. 2-81)

Project Description

Objectives:

A major objective is to provide consultative services in statistical and epidemiological methodology for the design, conduct, interpretation and evaluation of randomized clinical trials. This objective includes providing full statistical support for some major clinical trials of cancer treatment. For example, we are currently involved in studies of lung, breast, testis, and brain cancer. A second major objective is to provide consultation and scientific collaboration with investigators conducting other types of studies requiring statistical expertise. Some current examples include a study of the psychological aspects of breast cancer, an evaluation of hospice care for patients with terminal cancer, analysis of data from a registry of patients with mycosis fungoides, and non-randomized comparisons of radiotherapy with endocrine treatment for patients with prostate cancer.

Methods Employed:

Standard methods of biometry, statistics, probability, epidemiology, and computing techniques with necessary modification as required by the particular problem. New techniques are developed by the personnel working in the Section to handle specific problems (see Project No. Z01-CP-04409-06-B).

The most important projects of the Section will be described separately. Generally, in consultations on clinical trials, members of the Section assist the investigators in developing detailed study protocols, in determining the numbers of patients necessary for the study, in deciding what data should be recorded and at what intervals in time, and in developing forms for the recording of data. They advise on proper methods of analysis of the final data or undertake these analyses themselves. Such assistance is generally acknowledged in the publication of findings by the medical investigators.

Prostate Cancer:

Carefully collected data on over 4000 patients with prostate cancer were obtained from the randomized clinical trials conducted by the Veterans Administration Cooperative Urological Research Group (VACURG) between 1960 and 1975. In the last year, Dr. Byar and Mr. Corle have published a final report of a randomized comparison of prostatectomy plus placebo versus placebo alone in treating 121 patients with stages I and II prostatic cancer. Only 5 patients have died of prostatic cancer and rates for progression (defined as occurring at first rise in prostatic acid phosphatase, appearance of metastases, or death from prostatic carcinoma) did not differ significantly between the two treatments. They also demonstrated that the level of the prostatic acid phosphatase, even in the normal range and in patients whose prostate had been removed, was prognostic for progression. These results taken together suggest that cancer of the prostate, like breast cancer, metastasizes early in some patients and that some systemic therapy should be used in addition to or instead of surgery.

Also in the last year, Mr. Corle and Dr. Byar have completed a final analysis of the third VACURG study in which both estrogens and progesterone were used alone and in combination to treat patients in stages III (local extension) and IV (distant metastasis). No significant differences in survival were detected. The combined treatment caused a more marked fall in the serum testosterone, but this effect was not reflected in the comparisons of survival or tumor progression.

Dr. Byar is using the VACURG data for patients treated with hormones or orchiectomy in comparisons of survival, adjusted for stage and grade, with data from three centers (Stanford, Calif., St. Louis, Mo., and Boston, Mass.) where similar patients were treated by x-ray therapy. No large-scale randomized clinical trial has been completed comparing x-ray therapy to any other form of treatment, so these non-randomized comparisons will be important in assessing the role of irradiation in treating this disease.

Bladder Cancer:

In 1977, Dr. Byar and others reported the result of a VACURG study of treatment for stage I bladder cancer (recurrent papillomas) in which 25 mg daily oral pyridoxine (vitamin B6) was compared to placebo, and to monthly topical installation of thiotepa. These data suggested that pyridoxine might be helping some patients. Subsequently Dr. Byar helped design a second trial comparing only placebo and pyridoxine, to be conducted by the Genito-Urinary Group of the EORTC. In this trial, unlike its predecessor, tryptophane load tests would be performed before and during treatment because the experimental evidence suggesting the use of pyridoxine indicated that it might work by suppressing the levels of urinary metabolites of tryptophane which have been shown to cause bladder cancer in mice. By November 1980 this trial had accessioned some 200 patients and entry is continuing. Dr. Byar is assisting the EORTC statisticians in monitoring this trial, and he periodically analyzes the data independently.

Testicular Cancer:

Dr. Green is responsible for study design, implementation and analysis of the Intergroup Study of Testicular Cancer, a nationwide randomized trial comparing adjuvant combination chemotherapy following surgery for resectable stage II disease versus using chemotherapy only for relapses. This study is also following stage I patients to determine factors which may predict which tumors will recur. By April 1981, 99 patients had been admitted to the randomized trial and 79 stage I patients were being followed. Patient accrual is continuing and data are being systematically edited.

Data Center for Breast Cancer Studies:

The current files contain data on 3000 women with breast cancer who have also had estrogen receptor assays performed. Analyses by Mr. Corle and Dr. Byar have revealed that estrogen receptor values have prognostic importance for recurrence of disease and are correlated with other prognostic indicators. Detailed chemotherapy treatment information was added for 391 patients with advanced disease. Analyses of these data will be aimed at defining the importance of estrogen receptor in predicting response to chemotherapy. Also,

serum and background data have been collected from nearly 10,000 women for evaluation of biological markers for breast cancer in cooperation with the Markers Group of the Breast Cancer Task Force. Several panels of sera have been sent out for analysis, with the frequency of requests increasing near year's end. The Section is responsible for the collection, editing, and analysis of all data, and for providing a continually updated inventory of material in the serum bank. This project will be continued for a number of years.

Psychological Aspects of Breast Cancer:

Dr. Muenz is chief statistician for a large prospective epidemiological study designed to assess psychological consequences of mastectomy. Data collection on that study has now ended; 1713 women in four study groups (mastectomy and three control groups) have responded to questionnaires and received ratings indicating their emotional state at various times during the year following surgery. The analyses of these data are complete with the following conclusions: 1) There is a consistent pattern of enhanced distress among those with stage II (node positive) breast cancer compared to those in stage I (node negative). After that, in order of decreasing distress are found the cholecystectomy group (nearly tied with the stage I group), then the benign biopsy and healthy controls at low, nearly equal levels. This ordering holds for various measures of distress. 2) The differences, while highly significant, are often very small. 3) There is little change in distress over the course of the year following surgery, i.e., the psychological instrument scores remain nearly constant. 4) Higher education/income and older age contribute to diminished distress.

Inflammatory Cancer of the Breast in Tunisia:

Work on the extremely aggressive form of inflammatory breast cancer found in Tunisia has continued, with Dr. Muenz as the principal statistician. A paper on the epidemiology of this disease has been published. Entry into a randomized clinical trial has closed; three years' follow-up confirms the impression that chemotherapy is highly effective in reducing tumor volume, and that there is little difference in two modes (radiotherapy or surgery) of local tumor control. There is also little evidence that chemotherapy administered before local treatment is effective in prolonging the time until distant dissemination.

A Cooperative European Study of Surgery with and without Radiotherapy for Stage II Breast Cancer:

Since its inception in 1974, Dr. Muenz has been the chief statistician for a prospective randomized trial of breast cancer therapy conducted by the Ludwig Cancer Institute (Berne, Switzerland) in cooperation with the IARC. There has now been an average of three years of follow-up for the most recently admitted of the 397 patients in this study and the following conclusions have emerged: 1) There is no significant difference between the two treatment groups for time to distant metastasis or death. 2) There is a large and highly significant difference favoring radiotherapy for time to local (chest wall or supraclavicular node) recurrence. 3) The benefit of radiotherapy was confined

to patients with poorly differentiated large tumors or with tumors close to the internal mammary chain.

Isotopic bone scans are done repeatedly in this study. About 40% of the patients have developed distant metastases and these data will allow a statistical analysis of the value of the isotopic scan to detect metastases early compared to repeated x-ray examinations.

Trials for Lung Cancer:

Drs. Gail and Rubinstein are statisticians for the Lung Cancer Study Group, which is comprised of six major centers with the capacity to recruit over 150 stage I lung cancer patients per year. Five prospective randomized trials are in progress. Dr. Gail is responsible for three protocols. The first, a double-blind trial of intrathoracic BCG immunotherapy versus conventional therapy in stage I patients with resected disease has nearly completed its accrual phase, with over 473 patients on study. An analysis of intrathoracic BCG toxicity has been published and a report on the early effects of BCG treatment on recurrence and survival and on the usefulness of immunological parameters for prognosis and for monitoring these patients is being prepared. The second study compares radiotherapy and conventional therapy in stage II/III resectable patients with squamous cell disease. The third study compares a regime of cis-platinum, adriamycin and cytoxan (CAP) with BCG plus Levamisole in stage II/III patients with resectable adenocarcinoma or large cell carcinoma. Dr. Rubinstein is responsible for two protocols. The first compares CAP plus radiotherapy and radiotherapy alone in patients with partially resected non-small cell lung cancer. The second study compares CAP therapy with placebo in the subset of stage I patients with T1N1 or T2N0 disease. In addition, Dr. Rubinstein is surveying reasons for non-entry into present Lung Cancer Study Group trials in an effort to improve accrual and is studying characteristics of the available patient population for accrual into future trials. He is also maintaining a registry and natural history catalog of stage I patients with T1N0 disease.

Brain Tumor Clinical Trials:

Drs. Green and Byar are working extensively with the Brain Tumor Study Group on design and analysis of a number of large-scale randomized clinical trials. The first study analyzed (BTSG 72-01) showed improved survival when radiotherapy was added to surgery for malignant glioma and indicated a modest further benefit from nitrosourea chemotherapy. Of particular importance in this analysis was the identification of important prognostic factors and their use for adjustment of treatment comparisons. The second study analyzed (BTSG 75-01) showed that Procarbazine was equivalent to BCNU for the treatment of malignant glioma, but high-dose steroids did not improve survival compared to the conventional doses of steroids used in treating cerebral edema. The analysis of prognostic variables was extended and additional factors were identified. In addition, Drs. Green and Byar have been analyzing a series of phase II trials for brain tumors (both primary and metastatic). Planning and design efforts have involved two new trials: a phase III trial to investigate combined and sequential chemotherapy, which began accrual of patients in November 1980, and a phase II trial of new chemotherapeutic agents which is

scheduled to begin in late 1981.

Mycosis Fungoides:

In the past two years Drs. Green and Byar, in collaboration with Dr. Stanford Lamberg of Johns Hopkins University, analyzed prognostic factors in patients from 20 collaborating institutions registered by the Mycosis Fungoides Cooperative Group. Dr. Byar is currently completing this project by analyzing

the prognostic importance of histological features seen in biopsy specimens, adjusting for the effects of other variables.

Hospice Care for Terminal Patients:

Dr. Muenz is statistician for a cooperative pilot study designed to develop test procedures and gain experience for a future study of hospice care for terminal cancer patients. This study assesses the psychological state of hospice patients and their immediate families, and measure attitudes of hospice staff concerning terminal care. New patient entry closed on June 30, 1980. About 160 patients have been followed for a median survival of one month since hospice entry. Substantial amounts of information have been collected regarding the relatives of patients and their response to the "family-oriented" program of each of the three participating hospices. Dr. Muenz and other study participants are writing a book on the now completed study.

Makari Skin Test:

Dr. Levin is serving as statistician for two studies of the Makari Skin Test. The first evaluates the test as an aid to diagnosis, and the second will determine the prognostic value of the test in predicting subsequent recurrence of disease after surgical resection of colorectal, lung, and breast cancer. These studies, sponsored by the Stauffer Chemical Company, are being conducted in several collaborating medical centers in the U.S. and in England with the assistance of Dr. Ronald Herberman, Chief, Laboratory of Immunodiagnosis, NCI.

Burkitt's Lymphoma Project:

Dr. R. Bigger, of the NCI Environmental Epidemiology Branch, and Dr. Gail are analyzing records from 388 patients with Burkitt's lymphoma who were treated in Ghana since 1966. The results suggest that a simple staging system based on extent of disease is of prognostic value and that there has been a slight improvement in survival, adjusted for stage of disease, since the inception of the program. Other prognostic factors including therapy, age, sex and duration of prior symptoms have also been studied.

Neurofibromatosis:

In conjunction with Dr. Judith Bader of the NCI Clinical Epidemiology Branch, Dr. Muenz is acting as statistician for studies of neurofibromatosis designed to investigate the influence of father's age on incidence and the possible impairment of speech quality due to hypoglossal and glossopharyngeal nerve involvement. The paternal age issue will be examined by comparison with

unaffected siblings and with census data. Speech quality will be assessed by ratings of taped speech samples from affected and unaffected persons.

Human Chorionic Gonadatropin:

The Food and Drug Administration wishes to standardize criteria for the certification of home pregnancy-test kits which employ a quantal assay of HCG in urine. A multi-institution, multi-sample experiment has been performed to test, under ideal conditions, the sensitivity and specificity of the technique which, in simplified form, is used in the kits. Dr. Muenz is statistician for this study which also examines the use of HCG as a liver cancer marker.

Breast Cancer Skin Test Antigens:

Dr. Levin served as statistician on a study by Dr. Faye Austin, of the NCI Laboratory of Viral Carcinogenesis, evaluating the effect of virus augmentation of cultured breast tumor cell lines on the sensitivity and specificity of skin tests using antigens derived from the tumor cells.

Intra-Ocular Melanoma:

Dr. Levin is collaborating with Dr. Peggy Tucker of the NCI Environmental Epidemiology Branch on a study to identify prognostic factors in intra-ocular malignant melanoma.

Other Consultative Activities:

Dr. Byar serves on the NCI Chemoprevention Working Group. This group advises the Board of Scientific Counselors of DRCCA, NCI, on scientific matters relating to chemoprevention.

Dr. Gail and Dr. McIntire, of the NCI Diagnosis Branch, are organizing a symposium at which statisticians from several centers will present analyses of the same problematic data sets. The aim of this symposium is to improve quantitative methods for the design and analysis of clinical protocols for evaluating immunodiagnostic tests as aids to diagnosis, prognosis, and monitoring of disease. Emphasis will be on finding ways to combine several immunologic tests to produce optimal discrimination for diagnosis.

Dr. Gail, in collaboration with Dr. Petrone at Georgetown Medical School, has analyzed ankle injury data on a series of 150 patients to investigate the prognostic significance of age, site of injury, severity of the injury, nature of the reduction, and other factors.

Dr. Gail is collaborating with Professor Phillis Brown of the University of Rhode Island to develop methods to analyze nucleoside spectra from high pressue liquid chromatography. These spectra, which are obtained from serum samples, may have potential for discriminating cancer patients from those with benign disease.

Dr. Rubinstein is assisting Dr. Jeffrey Arbeit of NCI in analyzing a study of alanine to glucose conversion in tumor-bearing and non-tumor-bearing rats.

Dr. Levin has completed his work through the Office of International Affairs on collaborative studies of cancer epidemiology with the Soviet Union. He serves as American editor for a monograph entitled "Cancer Epidemiology in the U.S. and the USSR". He has participated in several scientific workshops concerned with evaluation of cancer epidemiology and design of large-scale studies to be conducted in the two countries.

Dr. Levin is serving as statistician on a study by Ms. Susan Fisher of the NCI Cancer Nursing Service to study the sexual knowledge and attitudes of professional nurses in the Cancer Nursing Service. The purpose of the study is to help prepare nurses for the problems associated with caring for cancer patients who face "particularly difficult psychosexual adjustment due to the disease and the current treatment modalities."

Dr. Gail continues to assist Dr. Ronald Herberman, of the NCI Laboratory of Immunodiagnosis, in the evaluation of new immunodiagnostic tests.

In collaboration with Dr. Stephen O'Brien of the Laboratory of Viral Carcinogenesis, Drs. Gail and Levin performed probabilistic calculations which demonstrate that the same enzymes tend to be polymorphic or monomorphic across species. In particular, they showed in cats, mice and humans, that the number of triply concordant enzymes, which are either polymorphic or monomorphic in each species, exceeds what would be expected by chance.

Dr. Green has consulted for the NCI Cancer Immunotherapy Program and has analyzed data from a Roswell Park study of adjuvant tumor-specific active immunotherapy of squamous cell carcinoma of the lung.

Dr. Green has consulted for Dr. Hugh Coakham, a visiting scientist at NINCDS, on an analysis of the relationship between presence or absence of antibody and survival of patients with malignant glioma.

Dr. Levin participated in a workshop sponsored by the International Union Against Cancer (UICC) in Geneva to prepare a UICC Technical Report on the Biology of Pancreatic Cancer.

Dr. Levin has continued to serve on the faculty of Georgetown University, helping to teach the freshman course on biostatistics in the Medical School.

Significance to Biomedical Research and the Program of the Institute:

The variability of the course of cancer in individual patients means that the assessment of treatment differences, determination of the usefulness of diagnostic tests, or the proper interpretation of data from observational studies are often statistical problems. Members of the Section are frequently consulted for advice or collaboration on such problems. Besides the projects listed above, there are numerous other short-term consultations dealing with specific studies, proposal reviews, site visits, and review of manuscripts submitted for publication. Some of the consultations involve extensive trials

which represent considerable efforts of the National Cancer Program. The Section represents an important resource for expert assistance in study design, implementation, and statistical and computer analysis of studies being carried out by many other groups.

In any healthy research environment, individuals skilled in many disciplines are necessary. The Section provides expertise in statistical matters relating to the study of cancer in humans. The ability to provide meaningful consultation is greatly enhanced by having four M.D.'s in the Section who are also well-equipped statisticians. Members of the section are also skilled in computer applications to analysis of medical data.

A further advantage to the Institute is that actual day-to-day experience as a statistical support center for several large-scale clinical trials and active involvement in consultation on other projects provides an ideal environment for identifying important methodological questions of general applicability to the design, conduct, and analysis of clinical trials.

Proposed Course of the Project:

In the coming year, we plan to continue our involvement in many of the studies described above. Major areas of emphasis will include the lung, testis, and brain cancer trials being conducted by the DCT.

Publications:

Austin, F.C., Boone, C.W., Levin, D.L., Cavins, J.A., Case, R., Pharm, M. and Klein, E.: Breast cancer skin test antigens of increased sensitivity prepared from vesicular stomatitis virus-infected tumor cells. Cancer, in press.

Blackman, M.R., Weintraub, B.D., Rosen, S.W., Kourides, I.A., Steinwasher, K., and Gail, M.H.: Human placental and pituitary glycoprotein hormones and their subunits as tumor markers: A quantitative assessment. J. Natl. Cancer Inst. 65: 81-93, 1980.

Byar, D.P.: VACURG studies of conservative treatment. Scand. J. Urol. and Nephrology 55: 99-102, 1980.

Byar, D.P.: VACURG studies of post-prostatectomy survival. Scand. J. Urol. and Nephrology 55: 113-116, 1980.

Costa, J., Webber, B.L., Muenz, L., O'Conor, G.T., Tabbane, F., Belhassen, S., Kamaraju, L.S., Mourali, N., and Levine, P.H. Histopathological features of rapidly progressing breast carcinoma in Tunisia: A study of 158 cases. International Journal of Cancer, in press, 1981.

Gail, M.H., Beach, J., Dark, A., Lewis, R. and Morrow, H.: A double blind randomized trial of low dose oral urea to prevent sickle cell crises. J. Chronic Diseases, in press.

Gail, M.H., Oldham, R.K., Holmes, E.C., et al.: Early side effects of intrathoracic BCG therapy in patients with Stage I squamous cell carcinoma, adenocarcinoma, or large cell lung cancer. Cancer Immunology and Immunotherapy 10: 129-137, 1981.

Green, S.B., Byar, D.P., and Lamberg, S.I.: Prognostic variables in mycosis fungoides. Cancer, in press 1981.

Levin, D.L. (Ed.): Cancer Epidemiology in the USA and the USSR. U.S. Department of Health and Human Services. Publication No. (NIH) 80-2044. Washington, D.C., U.S. Government Printing Office, July 1980.

Levin, D.L., Connelly, R.R., and Devesa, S.S.: Demographic characteristics of cancer of the pancreas: Mortality, incidence, and survival. Proceedings of the International Meeting on Pancreatic Cancer, New Orleans, La., March 10-11, 1980, Cancer 47: 1456-1468, 1981.

Mourali, N., Muenz, L., Tabbane, F., and Levine, P.: Epidemiological factors in Tunisian women with rapidly growing breast cancer. Cancer 46: 2741-2746, 1980.

O'Brien, S.J., Gail, M.H. and Levin, D.L.: Correlative genetic variation in natural populations of cats, mice and men. Nature 228: 580-583, 1980.

O'Brien, S.J., Shannon, J.E., and Gail, M.H.: A molecular approach to the identification and individualization of human and animal cells in culture: Isoenzyme and allozyme genetic signatures. In Vitro 16: 119-135, 1980.

Tal, C., Herberman, R.B., Gail, M.H., and Schutt, A.: Cancer associated serum globulin, demonstrated by polyacrylamide gel electrophoresis. J. Natl. Cancer Inst. 64: 1007-1014, 1980.

Walker, M.D., Green, S.B., Byar, D.P., et al.: Randomized comparisons of radiotherapy and nitrosoureas for the treatment of malignant glioma after surgery. N Engl. J. Med. 303: 1323-1329, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04265-16 B

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Consulting in Statistics and Applied Mathematics

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I. :	J.J. Gart	Head, Mathematical Statistics & Applied Mathematics Section, Mathematical Statistician	BB	NCI
	H. M. Pettigrew	Mathematician	BB	NCI
	R. E. Tarone	Mathematical Statistician	BB	NCI
	D.G. Thomas	Mathematical Statistician	BB	NCI
	J. Nam	Mathematical Statistician	BB	NCI
Other:	A. M. Smith	Statistician (Health)	BB	NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Biometry Branch, Field Studies and Statistics Program

SECTION
Mathematical Statistics and Applied Mathematics Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	3.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It is the purpose of this study to collaborate with NCI researchers on mathematical problems related to many areas of cancer research. Consulting assistance in statistical methodology and applied mathematics is provided for NCI investigators and to some extent for NCI contractors. In general, the study is devoted to accelerating the use of quantitative methodology in various aspects of the NCI intramural and extramural programs.

PHS-6040
(Rev. 2-81)

Project Description

To collaborate with NCI scientists on the applications of mathematical statistics and applied mathematics to the design, analysis, and interpretation of scientific experiments.

Objectives:

The principal objectives are (1) to collaborate with NCI scientists on mathematical problems related to cancer research, (2) to provide consulting assistance in statistics and applied mathematics to NCI investigators, and (3) to accelerate the use of quantitative methodology in various aspects of the NCI intramural program and extramural program.

Methods Employed:

The methodology of applied mathematics, mathematical statistics and probability is applied to biomedical problems. Often variations of existing techniques are developed to suit the special requirements of a particular problem.

Major Findings:

During this year, the staff advised and collaborated with many investigators in the major divisions of research in the National Cancer Institute as well as some outside contractors and other government agencies. The various projects are grouped below in terms of the divisions and areas of the projects.

Office of the Director - NCI

Dr. Pettigrew confirmed for Dr. Bayard H. Morrison the published results of a clinical trial conducted at the Mayo Clinic which failed to find any significant benefit of high-dose vitamin C in patients with advanced cancer. He advised the NCI Office of Cancer Communications on a response to Dr. Linus Pauling correcting his interpretation of statistical significance tests.

Division of Cancer Cause and Prevention - Office of the Director

Dr. Tarone continued to collaborate with Dr. Thomas Cameron on a paper detailing the results of a study comparing survival, prostate tumor rates and breeding efficiencies of two strains of rats.

Division of Cancer Cause and Prevention - Field Studies and Statistics

Dr. Gart is collaborating on two large prospective studies on the relationship of diet and cancer. One study done under contract at the University of Minnesota investigates deaths over an 11-1/2 year period among a cohort of men in a life insurance plan. Much of the analyses of this study was

formulated by Dr. Gart and implemented on the computer by Mr. Thomas and Mrs. Smith. With Professor L. Schuman and Professor E. Bjelke, Dr. Gart is co-author of two papers being submitted for publication. One documents the negative association between lung cancer and a vitamin A index, particularly as it reflects the consumption of vegetables. A second paper investigates the association of cancer at several sites with the use of alcohol. An indication is found, for a small number of such deaths, that pancreatic cancer is associated with its use, but there appears to be no connection with coffee use. The second study concerns the incidence of cancer among a similar cohort in Norway. Some of these data were analyzed by Mr. Thomas using the elaborate computer program he wrote for prospective studies with losses. With Professor Bjelke of The University of Bergen, Dr. Gart is co-authoring two parallel papers on diet and lung cancer as well as alcohol and cancer at several sites. In both these contracts, Mr. Scotto, of the Biometry Branch, serves as the project officer and contributes effectively to the performance of the research.

Dr. Tarone advised Ms. Margot Hanson of the Clinical Epidemiology Branch concerning the derivation of variance estimators for various age-adjustd rates. He also advised Dr. Elisabeth McKeen of the Clinical Epidemiology Branch concerning the analysis of data obtained from matched pairs.

Mr. Nam continues to collaborate with Mr. Joseph Scotto of the Biometry Branch on the statistical analysis of non-melanoma cases from the Skin Cancer Incidence Data System.

Mr. Thomas maintained and updated the section's library of computer routines which are often used in the consulting activities of other section members. Again this year, numerous computer installations throughout the world have requested and received copies of the programs developed and used in this section.

Mrs. Smith did much of the data processing and support work for many of the consulting projects detailed herein.

Dr. Gart continued to serve on the FS&S Program Review Group.

Division of Cancer Cause and Prevention - Carcinogenesis Intramural Program

Dr. Tarone continued to assist Dr. Umberto Saffiotti, Mr. Paul Donovan, and Dr. Enrico Cortesi of the Laboratory of Experimental Pathology with the design and analysis of bacterial mutagenesis assays and mammalian cell transformation assays to study the combined effect of mixtures of low doses of known mutagens.

Dr. Pettigrew has continued to provide statistical consultation on the analysis of experimental data obtained under NCI contract by Dr. Albert Segaloff of the Alton Ochsner Medical Foundation, New Orleans, Louisiana. A manuscript reporting the results of an experiment to investigate the apparent protective effect of early pregnancy on mammary tumor incidence

has been completed and is to be submitted for publication. Additional reports of data from experiments to study the effects of fractionated doses on the synergism between radiation and estrogen administration in mammary carcinogenesis are being prepared.

Mr. Nam is collaborating with Dr. Richard Yamamoto of the Laboratory of Carcinogen Metabolism on a study of mutagenicity of urine from rats after administration of Diaminoanisole (DAA) and Tyrosine to evaluate Tyrosine as inhibitor of mutagenesis. Mr. Nam continues to advise Dr. Yamamoto on the design and statistical analysis of laboratory experiments.

Dr. Tarone continues to collaborate with Dr. Kenneth Kraemer of the Laboratory of Molecular Carcinogenesis in studies on the effects of in vitro exposure to 8-methoxypsoralen and ultraviolet light on human lymphoid cells, and in a study of aryl hydrocarbon hydroxylase induction levels in psoriasis patients. Dr. Tarone is also collaborating with Dr. Kraemer on a study examining the survival of lymphoid cells from ataxia telangectasia patients and their related heterozygotes after exposure to bleomycin.

Mr. Nam and Dr. Gart are collaborating with Dr. Paul Levin of Carcinogenesis Intramural Program in a study of the relation between HLA (human leukocyte antigen) system and survivorship among Chinese nasopharyngeal carcinoma (NPC) cases in Singapore, as well as association between Epstein-Barr virus and antibodies and survivorship among NPC cases.

Dr. Tarone continues to work on the design and statistical analysis of experiments performed by Dr. Katherine Sanford, Dr. Raymond Gantt and Mr. Gary Jones of the Laboratory of Cellular and Molecular Biology and Dr. Ram Parshad of the Howard University College of Medicine. These experiments are performed to investigate factors which influence fluorescent light-induced chromosome damage in human and rodent cells in culture, and to attempt to explain the increased susceptibility to light-induced damage in malignant cell lines. Dr. Tarone also assisted Dr. William Taylor of the Laboratory of Cellular and Molecular Biology with the design and analysis of factorial experiments to study factors which influence the growth of human cells in culture.

National Toxicology Program

Dr. Tarone continued to collaborate with Dr. Jerrold Ward of the Tumor Pathology Branch on a paper detailing the extent of within and between laboratory variation in control tumor rates for various organs in B6C3F1 mice and Fischer 344 rats used in carcinogenesis bioassay experiments.

Dr. Tarone continued to assist Dr. Kenneth Chu of the Technical Resources Branch and Dr. Virginia Dunkel of the Food and Drug Administration in writing a paper concerning the reproducibility of microbial mutagenicity assays. Dr. Tarone also advised Dr. Chu concerning methods of analyzing standardized mortality ratios.

Division of Cancer Biology and Diagnosis

Mr. Nam and Dr. Gart are collaborating with Dr. Stephen Shaw of the Immunology Branch on a case-control study of the association between Dermatitis Herpetiformis disease and the Second B cell antigen (SB) locus of the HL-A system.

Dr. Pettigrew assisted Drs. Pietro Gullino and Marina Ziche of the Laboratory of Pathophysiology in the preparation of a manuscript reporting the results of an experiment investigating the ability of fragments from plastic cover slips that had been inserted subcutaneously in mice for from 1 week to 16 weeks to stimulate angiogenesis in rabbit corneas.

Dr. Tarone continued his collaboration with Dr. Jay H. Robbins, Dr. Alan N. Moshell, Dr. Ronald G. Scarpinato and Ms. Susanna Barrett of the Dermatology Branch in their experiments to study the in vitro survival of lymphocyte and fibroblast cell lines from patients with the cancer prone disease, xeroderma pigmentosum, and other hereditary primary neuronal degenerations after exposure to the DNA-damaging agents such as ultraviolet light, X-ray, and MNNG.

Division of Cancer Treatment

Dr. Tarone continues to provide statistical consultation to Dr. Louis Hodes of the Pharmaceutical Resources Branch concerning methods of predicting the pharmacological activity of potential anti-cancer drugs based on their chemical structure.

Various Other Activities

Dr. Tarone and Dr. Gart are collaborating on the writing of two chapters for an International Agency for Research on Cancer monograph on the statistical analysis of long-term animal carcinogenesis experiments.

Dr. Pettigrew is working with Drs. Yale Topper and Takami Oka of NIAMD in studies with obese mice concerning the effects of insulin analogs on glycogen synthesis in diaphragms and on hepatic ODC activity.

Dr. Gart has completed his service on two important working groups involved with the Bureau of Foods of the FDA. One is the Interagency Working Group on Nitrite Research. The final report, which used statistical analysis for animal testing developed mainly in this section, was issued in late 1980. Dr. Gart also served on the FDA's FD&C Red No. 40 Working Group. He directed and wrote much of the statistical analyses contained in the report, a good deal of which employs methods developed in this section. He is preparing at least one paper detailing some of the statistical issues involved for publication.

Dr. Pettigrew continued his service on the National Research Council, Assembly of Life Sciences, Board on Toxicology and Environmental Health

Hazards Committee on Alkyl Benzene Derivatives, which produced a report entitled the Alkyl Benzenes for the Office of Research and Development of the U.S. Environmental Protection Agency.

Dr. Pettigrew continues to serve as a member of the Interagency Regulatory Liaison Group (IRLG) to review the NCTR/FDA ED study.

Dr. Tarone served as an expert consultant to the Scientific Panel of the Interagency Work Group on Phenoxy Herbicides and Contaminants in the review of a mouse teratogenicity study of various combinations of the chemicals, 2,4-D, 2,4,5-T and TCDD, found in the herbicide, Agent Orange.

Dr. Tarone served as an expert consultant to the Federal Insecticide, Fungicide, and Rodenticide Act Advisory Panel of the Environmental Protection Agency in a meeting to evaluate the carcinogenic potential of the pesticide, Permethrin.

Dr. Pettigrew is assisting the Office of the General Counsel, Food and Drug Administration, in developing evidence on issues relating to the carcinogenic potential of furazolidone, a substance used in chicken and swine feed.

Miscellaneous

Dr. Tarone performed the statistical analysis of data from a rodent carcinogenesis bioassay of the chemical (Di-2-ethylhexal) Phthalate (DEHP) for Dr. Harry Milman in the Office of Testing and Evaluation of the Environmental Protection Agency.

Refereeing

Dr. Pettigrew refereed for Cancer. Mr. Nam, Dr. Tarone, and Dr. Gart refereed for the Journal of the National Cancer Institute. Mr. Nam refereed for Cancer Research, Dr. Tarone refereed for the Journal of Educational Statistics and Dr. Connor refereed for the American Journal of Public Health.

Significance to Biomedical Research and the Program of the Institute:

Members of this section are assuming an essential role in much research within the National Cancer Institute. Their activities include not only statistical analysis but also planning of valid experiments.

Proposed Course:

Several of the projects mentioned in the Major Findings section will continue. In particular, the collaboration with the various projects in epidemiology within Field Studies and Statistics, as well as in the Carcinogenesis Research Area, the Division of Cancer Biology and Diagnosis, and other areas will be progressing.

Publications

Barrett, S.F., Tarone, R.E., Moshell, A.N., Ganges, M.B., and Robbins, J.H.: The post-UV colony forming ability of normal fibroblast strains and of the xeroderma pigmentosum group G strain. J. Invest. Dermatol. 76: 59-62, 1981.

Chu, K.C., Patel, K.M., Lin, A.H., Tarone, R.E., Linhart, M.S., and Dunkel, V.C.: Evaluating statistical analyses and reproducibility of microbial mutagenicity assays. Mutation Research, in press.

Goldstein, K., Lai, P.K., Lightfoote, M., Andrese, A.P., Fuiccillo, D., Connor, R.J., and Levine, P.H.: Relationship of in vitro immune responses to Epstein-Barr herpesvirus and the severity of infectious mononucleosis. Infection and Immunity 29: 945-952, 1980.

Hayes, H.M., Hoover, R., and Tarone, R.E.: Bladder cancer in pet dogs: A sentinel for environmental cancer? Am. J. Epidemiol., in press.

Kraemer, K.H., Levis, W.R., Cason, J.C., and Tarone, R.E.: Inhibition of mixed leukocyte culture reaction by 8-methoxypsoralen and long wavelength ultraviolet radiation. J. Invest. Dermatol., in press.

Kraemer, K.H., Waters, H.L., Cohen, L., Popescu, N.C., Amsbaugh, S.C., DiPaolo, J.A., Glaubiger, D., Ellingson, O.L., and Tarone, R.E.: Effects of 8-methoxypsoralen and ultraviolet radiation on human lymphoid cells in vitro. J. Invest. Dermatol. 76: 80-87, 1981.

Moshell, A.N., Tarone, R.E., Newfield, S.A., Andrews, A.D., and Robbins, J.H.: A simple and rapid method for evaluating the survival of xeroderma pigmentosum lymphocyte lines after irradiation with ultraviolet light. In Vitro, in press.

Nam, J., and Scotto, J.: Letter to Editor. Am. J. Epidemiol. 113: in press, 1981.

Parshad, R., Sanford, K.K., Jones, G.M., Tarone, R.E., Hoffman, H.A., and Grier, A.H.: Susceptibility to fluorescent light-induced chromatid breaks associated with DNA repair deficiency and malignant transformation. Cancer Research 40: 4415-4419, 1980.

Parshad, R., Taylor, W.G., Sanford, K.K., Camalier, R.F., Gantt, R., and Tarone, R.E.: Fluorescent light-induced chromosome damage in human IMR-90 fibroblasts: Role of hydrogen peroxide and related free radicals. Mutation Research 73: 115-124, 1980.

Perlin, E., Oldham, R.K., Weese, J.L., Heim, W., Reid, J., Mills, M., Miller, C., Blom, J., Green, D., Bellinger, Jr., S., Cannon, G.B., Law, I., Connor, R., and Herberman, R.B.: Immunotherapy of carcinoma of the lung with intradermal BCG and allogeneic cells. International Journal of Radiation Oncology, Biology and Physics 6: 1033-1039, 1980.

Perlin, E., Scialla, S.J., Connor, R.J.,: Blood coagulation profiles in patients with carcinoma of the lung. Military Medicine, in press.

Poirier, M.C., and Connor, R.J.: A radioimmunoassay for 2-acetylamino-fluorene-DNA adducts. In Van Vukinis, J., and Langone, J. (Eds.) Immunochemical Methods, Volume 4, New York, Academic Press, Inc., in press.

Reid, J.W., Perlin, E., Oldham, R.K., Weese, J.L., Heim, W., Mills, M., Miller, C., Blom, J., Green, D., Bellinger, S., Cannon, G.B., Law, I., Connor, R., and Herberman, R.B.: Immunotherapy of carcinoma of the lung with intradermal BCG and allogeneic tumor cells: A clinical trial. In Terry, W. (ed.) Immunotherapy of Cancer: Present Status of Trials in Man, New York, Raven Press, in press.

Stoner, G.D., Harris, C.C., Myers, G.A., Trump, B.F., and Connor, R.J.,: Putrescine stimulates growth of human bronchial epithelial cells in primary culture. In Vitro 16: 399-406, 1980.

Tarone, R.E., Chu, K.C., and Ward, J.M.: Variability in the rates of some common naturally occurring tumors in F344 rats and B6C3F1 mice. J. Natl. Cancer Inst., in press.

Ward, J.M., Frank, A.L., Wenk, M., Devor, D., Tarone, R.E.: Ingested asbestos and intestinal carcinogenesis in F344 rats. J. Environ. Pathol. Toxicol. 3: 301-312, 1980.

Weisburger, E.K., Ulland, B.M., Nam, J., Gart, J.J., and Weisburger, J.H.: Carcinogenicity tests of certain environmental and industrial chemicals. J. Natl. Cancer Inst., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04267-16 B

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Research in Mathematical Statistics and Applied Mathematics

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I. :	J. J. Gart	Head, Mathematical Statistics & Applied Mathematics Section Mathematical Statistician	BB	NCI
	H. M. Pettigrew	Mathematician	BB	NCI
	R. E. Tarone	Mathematical Statistician	BB	NCI
	D. G. Thomas	Mathematical Statistician	BB	NCI
	J. Nam	Mathematical Statistician	BB	NCI
Other:	A. M. Smith	Statistician (Health)	BB	NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Biometry Branch, Field Studies and Statistics Program

SECTION
Mathematical Statistics and Applied Mathematics Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	3.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It is the purpose of this project to conduct research in mathematical statistics, probability and applied mathematics, and especially to develop new statistical methodology which is particularly applicable to the biomedical sciences. Particular subjects of interest are the methodology of analyzing survival curves and proportions, statistical methods in cancer epidemiology and statistical genetics, such as the analysis of the relative risk.

PHS-6040
(Rev. 2-81)

Project Description

Objectives:

To conduct research in mathematical statistics, probability, and applied mathematics; to develop new statistical methodology which is especially appropriate to biomedical sciences.

Methods Employed:

The methods employed are the modern theories of mathematical statistics, probability, and applied mathematics. High speed electronic computers are often used to compute appropriate mathematical tables and to test approximations by simulation techniques.

Major Findings:

The research of the members of this section covers a wide spectrum of topics in mathematical statistics, probability, and applied mathematics. These are summarized below.

John J. Gart and Donald G. Thomas completed and submitted a paper on the evaluation in the unconditional sample space, of three approximate confidence limits for the odds ratio. The three methods, widely used in case-control studies are Cornfield's method, the logit method with 1/2 corrections, and the test based method proposed by Miettinen. The last method, which has wide currency in the epidemiology literature is shown to underestimate the risk from a factor when such risk exists; and conversely, it exaggerates the estimated maximal risk when, in fact, no risk is present. The logit method also tends to underestimate the maximal risk when it is present. Cornfield's method is shown to be the clearly preferred method. Donald G. Thomas and John J. Gart are also implementing, via a computer program, an analysis of the odds ratios in prospective or cohort studies. The analysis is a stratified version of a previously published paper by these authors on trend and homogeneity analyses of proportions and life table data. In addition, univariate and bivariate logistic regression is incorporated in the methodology. This methodology has proven particularly useful in analyzing a prospective study of diet and cancer. A paper describing the methodology has been submitted for publication.

Robert E. Tarone developed an empirical Bayes method of incorporating historical control data into tests for trends in proportions. A paper based on this research has been accepted for publication. Robert E. Tarone continues his study of estimators which are approximations to maximum likelihood estimators. He has written a paper reporting simple estimators of summary relative risk for stratified data from prospective studies, and he is investigating simple estimators of hazard ratios for censored survival data. Dr. Tarone continues his investigations of optimal designs for evaluating synergism in data from microbial assays for mutagenicity,

distribution-free tests of censored distributions, and methods of computing simultaneous confidence intervals for several normal means when sample sizes are unequal. His joint research with John J. Gart on the application of score tests also continues.

Hugh M. Pettigrew is investigating models for tumor growth (such as the Gompertz function) and models for mammary tumor systems in rodents. His research in the mathematical theory of epidemics is continuing. He is also considering methods to compare linear trends in proportions in samples in which the observations are correlated, e.g., proportions of patients with metastases at a particular site vs. arterial blood flow to the site.

Jun-mo Nam and John J. Gart are continuing their research in statistical genetics on generalized ABO-systems. This work has particular relevance to analysis of data possibly linking the HLA (human leukocyte antigen) system and cancer. In addition to their paper in press on this general topic, they have developed a correction term to remove the bias in the one degree of freedom test of the Hardy-Weinberg law which is based on the simple Bernstein estimator.

Jun-mo Nam is also investigating a statistical method for detecting haplo-type association with diseases. He has also extended the computer program for analyzing HLA data to cases where only the antigen frequencies are available. Jun-mo Nam continues his research on sample size determination for a linear trend with proportions and on efficient statistical methods for identifying seasonal trends.

John. J. Gart has continued his research on several topics in statistical methodology. Among these are (1) investigation of the properties of the relative risk for case-control studies with multiple matched controls. The bias and relative efficiency of various proposed estimators are being calculated, and (2) investigation, with Hugh M. Pettigrew, of the higher order corrections to the mean and higher order moments of various transformations of binomial proportions. These include particularly the logit and logarithmic transformations which are routinely applied in so-called loglinear methods. In this work, Donald G. Thomas has done considerable computer programming of the exact calculation of the various moments.

John J. Gart has written an appendix to the National Research Council Report on a mathematical model which forms a theoretical basis for the "threshold limit" criterion for fixing permissible exposure levels for the mixture of possible harmful chemicals.

Alroy M. Smith provides computer programming support on many of the research projects in the section.

John J. Gart continues to serve as the Editor of Shorter Communications for Biometrics. In this capacity he is concerned with handling approximately one hundred manuscripts a year. Robert E. Tarone continues as an Associate Editor of the Theory and Methods Section of the Journal of the American Statistical Association. Jun-mo Nam, Robert E. Tarone, and

Robert J. Connor refereed for Biometrics. John J. Gart refereed for The Journal of the American Statistical Association. Robert E. Tarone and John J. Gart refereed for The American Statistician.

Significance to Biomedical Research and the Program of the Institute:

The interplay between mathematical theory, data analysis and experimental research is an important element in biomedical research. Many of the "major findings" reported above are new statistical techniques which have or may be directly applied to data collected by the medical researchers at NCI, particularly in DCCP, or other workers in cancer research. Others are mathematical models which may also aid in the planning of subsequent experiments or epidemiologic studies. The opportunity for initiating fundamental research on mathematics and mathematical statistics is essential for enabling members of the section to achieve professional recognition among their peers in their own scientific disciplines. More importantly, the possibility of doing such unconstrained research is a prerequisite for the consulting work of the section to be carried out at the highest professional level.

Proposed Course:

Many of the projects described in the major findings will be contined, e.g., analyses of transformations and loglinear methods, analyses of relative risk in case-control studies, research on censored survival tests, and statistical methods for teratogenic and mutagenic studies. In addition, new research initiatives will include the development of new statistical methods and mathematical models in various biomedical problems that come to our attention during the year.

Publications:

Gart, J.J.: Statistical analyses of the relative risk. Env. Health Persp. 32: 157-167, 1979.

Gart, J.J.: Mathematical models for chemical interactions. In Panel on Evaluation of Hazards Associated with Maritime Personnel Exposed to Multiple Cargo Vapors, National Research Council: Principles of Toxicological Interactions Associated with Multiple Chemical Exposures. Washington, D.C., National Academy Press, Appendix B, 1980, B1-B11..

Gart, J.J.: The identity of the Arbous-Kerrich "burnt fingers" distribution and Janardan's stochastic model for oviposition. Mathematical Biosciences, in press, 1981.

Gart, J.J. and Nam, J.: Statistical methods for genetic studies of HLA and cancer, In Cornell, R.G. (Ed.): Statistical Methods for Cancer Studies. New York, Marcel Dekker, Inc., in press, 1981.

Tarone, R.E.: On the distribution of the maximum of the log rank statistic and the modified Wilcoxon Statistic. Biometrics 37: 79-85.

Tarone, R.E.: The use of historical control information in testing for a trend in proportions. Biometrics, in press.

Tarone, R.E.: On summary estimators of relative risk. J. Chron. Dis., in press.

Tarone, R.E., and Gart, J.J.: On the robustness of combined tests for trends in proportions. J. Amer. Statist. Assoc. 75: 110-116, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04269 10 B

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)
Biomedical Computer Systems - Consultation, Research and Development, Service

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Stump	Acting Chief, Computer Science Section	BB	NCI
Other:	C. Hollingsworth	Computer Specialist	BB	NCI
	J. Larson	Supervisory Systems Analyst	BB	NCI
	V. Van Holten	Computer Specialist	BB	NCI
	R. Wolfson	Computer Programmer	BB	NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Biometry Branch, Field Studies & Statistics Program

SECTION
Computer Science Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland, 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
7.5	5	2.5

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is to facilitate the research activities and other operations of the National Cancer Program through the application of computer science, information technology, management science and operations research and by minimizing the organizational, operational and logistical constraints on scientific investigations and related activities which involve all aspects of data processing and computing, including collection, entry, purification, storage, computation, display and retrieval, analysis dissemination.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

The Computer Science Section (CSS) provides consultation and assistance to the scientific and administrative staff of the National Cancer Institute, and as necessary, to other governmental agencies, private institutions, and individual investigators who collaborate with the National Cancer Institute in its mission. Consultation and assistance are given on individual studies, as well as on multi-center studies, involving epidemiologic, laboratory and clinical investigations, cancer surveillance programs, cancer control programs, and information and reporting systems for cancer centers.

The Section conducts research and development work with computer scientists and specialists at universities and other institutions in order to develop new and improved methodologies in the application of computers to biomedical research.

Methods Employed:

Members of the Section apply systems analysis techniques to the planning, design, organization, administration, operation and evaluation of research projects having data management and statistical computing requirements. This technical assistance is provided through individual consultation and by directing the activities of computer systems analysts and programmers under contract to the National Cancer Institute.

The scope of the Computer Science Section consulting activities continues to be focused primarily on the Biometry Branch's scientific programs and studies. In addition to continuing the major initiative to design, develop and distribute uniform and standardized software routines to requesting registries in the Surveillance, Epidemiology and End Results (SEER) Program, members of the Section have increased consultation to Biometry Branch investigators on special studies. Another major Computer Science Section project this year involves the development and/or modification of special purpose generalized software (computer systems) that is required to initiate and carry out analytcal studies expeditiously, accurately and at minimal cost. Special emphasis is put on the implementation of user-oriented systems that allow investigators to be relatively independent of a systems analyst or computer programmer.

Major Findings:

The Surveillance, Epidemiology and End Results Program

This year the Section continued efforts to modify the core SEER Data Management System (SDMS) for implementation in the Connecticut Tumor Registry. Much of this year was devoted to refining system specifications and writing the computer routines to meet the unique requirements of the Connecticut Tumor

Registry. This project is under the overall direction of the Acting Section Chief and Valerie Van Holten serves as the principal analyst.

Due to the inability of the Louisiana Tumor Registry (LTR) to attract and retain qualified computer professionals, members of the Computer Science Section and staff of Geomet Technologies Inc., our computer support contractor, used the system that was developed for the LTR to process the backlog of cancer cases for 1979-80. Error reports were produced, the resulting corrections processed and a final masterfile and SEER extract tape were provided. Final implementation/operational documentation and a tape containing all computer routines were prepared and forwarded to LTR registry officials for implementation on their computers. This successfully concludes our consulting relationship with the Louisiana Tumor Registry.

The Computer Science Section continued to provide technical consultation to the Michigan Cancer Foundation on the development of a new data management system for the Detroit Area Tumor Registry. This year, Mr. James Larson provided specific consultation on the selection and acquisition of an in-house computer.

The Section continued to provide support services for processing the annual SEER submissions. Ms. Ruth Wolfson functioned as the interface between the SEER Program and each SEER registry on operational aspects of editing and updating each registry's data file. In addition, Ms. Wolfson contributed to a special SEER Quality Control study, modification of the SEER Information Retrieval System (SIRS) and processing of SEER compatible tape from the Israel Tumor Registry.

Other Biometry Branch Consultation

Mr. Larson provided assistance to Biometry Branch investigators on administration and monitoring of ADP support contractors and technical consultation on several studies including (a) Interview of Skin Cancer Patients in New Hampshire and Vermont, (b) Lutheran Brotherhood Dietary Study, (c) Norwegian Dietary Study, and (d) Incidence of Cancer in the U.S. among individuals with Spanish Surname. Ms. Wolfson provided computer programming support to several of these studies.

Mr. Calvin Hollingsworth provided consultation on a joint UCLA/NCI Chemical Mixture Study, and a special study of the smoking habits of World War II veterans. In addition, Mr. Hollingsworth was the lead analyst in streamlining the cost reporting system used to monitor the computer-related expenditures at DCRT.

Cancer Centers Program

Mr. Michael Stump continued to provide Cancer Centers Program officials with consultation in the review and analysis of the data management aspects of the Statistical Analysis and Quality Control (SAQC) Coordination Center contract. Mr. Stump chaired the contract selection committee to evaluate and to select a new SAQC contractor.

NTRA Workshops

The Section continues to participate in the Program of the National Tumor Registrar's Association (NTRA). This year Mr. Larson provided consultation to NTRA on the development of a federal position classification standard for tumor registrars. At the request of the Program Committee of NTRA, Mr. Larson was invited to the annual meeting in Williamsburg, Va., to discuss progress on the development of this standard.

Committee On Immunodiagnosis

Mr. Stump served as co-project officer on the computer support contract to the Committee on Immunodiagnosis. Mr. Stump and Ms. Helen Abbott functioned as the technical liaison between the contractor and Dr. Ronald Herberman, Chairman of the Immunodiagnosis Committee. CSS staff scheduled the analysis of data, approved the technical approaches proposed by the contractor and reviewed all completed analyses. In addition, Mr. Stump reviewed a proposal by staff of the Mayo Clinic to automate the Mayo Serum Bank.

Office of the Director, NCI

Frederick Cancer Research Center, EDP Working Group

Mr. Larson has been a member of the FCRC EDP Working Group for seven years. The group is responsible for monitoring the data processing portion of the Litton Bionetics FCRC contract. Mr. Larson's consultation at the FCRC is expected to continue at the same level.

Other Consultation

Mr. Larson served as a member of the contract selection committee for data processing support and analytical services for the National Institute of Mental Health's collaborative program on the Psychology of Depression.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04409-06 B

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Statistical Methodology Research

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. P. Byar	Chief, Clin. & Diag. Trials Section	BB NCI
OTHER:	M. H. Gail	Medical Statistical Investigator	BB NCI
	S. B. Green	Medical Researcher	BB NCI
	D. L. Levin	Senior Investigator	BB NCI
	L. R. Muenz	Mathematical Statistician	BB NCI
	D. K. Corle	Computer Systems Analyst	BB NCI
	L. V. Rubinstein	Staff Fellow	BB NCI
	E. V. Slud	Cancer Expert	BB NCI
	L. J. Wei	Cancer Expert	BB NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Biometry Branch, Field Studies & Statistics Program

SECTION
Clinical & Diagnostic Trials Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.0	5.0	1.0

CHECK APPROPRIATE BOX(ES)

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to conduct research in statistical methods and computer techniques with particular emphasis on those appropriate to the analysis of data from clinical and diagnostic trials and epidemiological studies of cancer. Many of the problems studied under this project arise from the consultative activities of the Section. In the past year this research has included new work on sequential analysis of clinical trial data in a variety of settings; methodological and computational work on time-dependent covariate analysis appropriate for studying the effects of serial tumor markers, total dose of chemotherapy, secondary treatments, and other sequentially recorded information; an investigation of various methods of constructing adjusted survival curves; and a study of multiple comparisons in two-way tables which may be helpful in interpreting results of subset analyses commonly performed in epidemiological studies.

PHS-6040
(Rev. 2-81)

Project Description

Objectives:

Many statistical problems arise in the consultative activities of the Section (see Project No. Z01 CP 04260-21 B). The investigation of these problems, the development of new statistical methods, and adaption of existing methods for special problems constitute an important aspect of the work of the Clinical and Diagnostic Trials Section. The basic objective of this work is to enrich the repertoire of statistical methods appropriate for the analysis of clinical trial data; therefore, emphasis is placed on statistical applications. However, statistical theory and application are so closely related that some of the work has a distinctly theoretical flavor. The research conducted under this project covers a wide spectrum of topics. Some of the principal subjects which have been studied during the past year are summarized below.

Sequential Monitoring of Clinical Trials:

Dr. Gail, in collaboration with Dr. DeMets of NHLBI and Dr. Slud, is examining the properties of group sequential tests applied to survival data to see whether proposed boundaries indeed yield the desired size and power, to determine how often they afford early trial termination, and to determine how their properties are affected by anomalies such as that which arises when sicker patients accrue first. This work includes studies on the correlation structure of logrank increments.

Drs. Slud and Wei have developed the asymptotic distribution theory of sequentially computed modified-Wilcoxon scores for two-sample survival data with random staggered entry and random loss to follow-up. They also applied this theory to a repeated significance testing procedure for testing the equality of two survival distributions, analyzing data on treatment of prostate cancer as an example.

Dr. Slud has also completed a theoretical study on sequential survival testing via partial-likelihoods concentrating on fully sequential tests with the logrank statistic. This study justifies the empirical results of Drs. Gail, DeMets and Slud, in showing under broad conditions that increments over time of logrank (Mantel-Haenszel) survival statistics are uncorrelated.

Accrual Stopping Rules for Clinical Trials:

Drs. Rubinstein and Gail have investigated circumstances under which one can monitor comparative survival studies with the logrank statistic and make an early decision to stop accrual without affecting the size or power of the fixed sample design. The basis of this proposal is the important distinction between a decision to stop accrual and a decision to test the statistical hypothesis. The latter can often be postponed until sufficient information is available.

Theory of Survival Analysis:

Dr. Slud has studied theoretically the consistency and efficiency properties of inferences from censored survival data using the partial-likelihood methods of Cox and of Peto and Peto.

Drs. Rubinstein and Slud are studying methods of analyzing survival data in the presence of censoring mechanisms which may not be independent of survival time for a given patient. Under various models of dependence for death and censoring times, they have developed estimators and two-sample tests for equality of survival curves.

Analysis of Time-Dependent Covariates:

Drs. Green and Byar have continued the investigation of methodology for analyzing covariates whose values change in time. In particular, they are studying how to measure the effect of a therapeutic intervention which may or may not occur at some time during the follow-up of a patient (other than at the time of randomization). Dr. Gail is extending previous work on serial tumor markers analyzed as time-dependent covariates to obtain absolute risk evaluations.

A Critique of Methods to Determine How Chemotherapeutic Effectiveness Depends on Total Dose Received:

Drs. Redmond and Wieand, University of Pittsburgh, and Drs. Gail and Byar are examining several techniques, including the use of a time-dependent covariate, to investigate possibly spurious claims that receiving the maximal protocol chemotherapeutic dose causes the patient to survive longer. Even the establishment of a reliable association requires careful examination of the data.

Adjusted Survival Curves:

Drs. Gail, Byar, and Green have continued their investigation of various methods of adjustment for survival curves, so that comparisons between different groups of patients take account of differences in patient covariates. Detailed study has been made of a new approach based on actuarial direct adjustment; this has been compared with saturated and unsaturated Weibull and Cox models, using both real clinical trial data and simulated examples.

Power Computations for Comparative Poisson Trials:

Dr. Green has collaborated with Dr. Brown of the Biometry Branch on an investigation of the power of comparative Poisson trials when there are unequal sizes of the populations being compared. These results are applicable for unbalanced sampling situations in studies investigating differences in the incidence of a rare disease or comparative binomial trials with very small binomial parameters.

Subset Analyses in Epidemiological Studies:

Dr. Slud has estimated by simulation the probability of turning up wholly spurious "significant" effects in case-control studies when analyzing subtables resulting from cross-classification by two binary variables (e.g., race and

sex). He and Dr. Byar are continuing this study in an effort to formulate general guidelines concerning subset analyses.

Likelihood Calculations for Matched Case-Control Studies and Survival Studies with Tied Death Times:

Drs. Gail and Rubinstein, in collaboration with Dr. J. Lubin of the NCI Environmental Epidemiology Branch, have developed recursive methods which permit exact conditional likelihood calculations for multiply matched case-control studies. These methods can also be used to solve partial likelihood equations in the Cox survival model with tied death times and to solve related problems in contingency table analyses. This work, which is to appear in Biometrika, renders current approximations unnecessary.

Confidence Limits for Estimates of Median Survival Time:

Drs. Slud and Byar are comparing by simulation the small sample performance of several methods of constructing confidence limits for estimates of median survival time obtained from censored and uncensored samples of sizes 20 and 40. In addition, Drs. Byar and Green have demonstrated that an actuarial approach given in a standard text on survival analysis may be grossly incorrect even in samples as large as 500.

Point and Interval Estimation of Location Difference for Fragmentary Samples:

Dr. Wei completed work concerning robust estimation on the difference between location parameters of correlated variables when some observations on either of the variables are missing. He showed that the point estimator is consistent, asymptotically normally distributed, and insensitive to outlying observations.

Nonparametric Estimation for a Scale-Change Model with Censored Observations:

Drs. Wei and Gail have studied the nonparametric point and interval estimators of the ratio of two scale parameters for arbitrarily right censored data based on the idea of Hodges and Lehmann. These estimators are defined in terms of rank statistics for testing the equality of two survival distributions. The asymptotic properties and efficiencies of estimators are also investigated.

Testing Symmetry and Independence in a Bivariate Distribution Function with Incomplete Paired Data:

Dr. Wei is working on a class of rank tests for testing the symmetry of a bivariate distribution function with incomplete paired data. The choice of score function is also being investigated. Drs. Rubinstein and Wei have developed a rank test for the independence of two variables when the paired data are incomplete. Simulation has shown it to be more efficient than the standard rank test utilizing only the complete pairs.

Randomization as a Basis for Data Analysis:

It is well known that unequal probability allocation (e.g., biased coin design, urn design, permuted block design) can bias tests based on the permutation

(equal allocation) distribution. Dr. Wei is investigating the asymptotic permutation distributions based on some restricted randomization schemes.

Asymptotically Distribution-Free Simultaneous Confidence Region of Treatment Differences in a Randomized Complete Block Design:

Dr. Wei has proposed and analyzed an asymptotically distribution-free simultaneous confidence region of pairwise treatment differences for a randomized complete block design with additive block effects. He showed that the corresponding confidence bound has an explicit form and is easily obtained.

Interactive Data Analysis Programs:

Dr. Green has continued to design, implement, and supervise improvements for a series of interactive computer programs for analyzing clinical trial data, and new programs have been incorporated into this package. This increases our capacity to respond efficiently to requests for sophisticated data analysis. These programs have been used increasingly for the specialized data analyses performed by members of the Section and by other members of the Biometry Branch.

Significance to Biomedical Research and the Program of the Institute:

Much of the work described above is directly applicable to the analysis of data collected in clinical trials of the treatment of cancer, analysis of data related to diagnosis and screening of cancer, and to epidemiologic studies of cancer. Beyond that, the opportunity to engage in research in statistical methods is a necessary and important aspect of the work of a consulting statistician. The prestige of the National Cancer Institute as a leader in biomedical research is enhanced by having on its staff statisticians of a high caliber who have made original contributions to their own discipline. Looking at it from another point of view, the opportunity for professional recognition by means of original research publications is essential in attracting to the Institute unusually talented statisticians and physicians interested in careers in biostatistics. Statistical theory and applied methodology is a rapidly expanding field. If the most appropriate methods are to be used in analyzing data related to cancer, then such individuals are essential to the program of the Institute.

Proposed Course:

Some of the separate projects described in this report have been completed, but some will be continued into the next year. In general the statistical research projects are suggested by problems which arise in our consulting work, so we anticipate that many new problems will be studied as they come to our attention in the next year. The Section will continue a program of balanced activities divided between research and consultation, theory and application.

Publications:

Byar, D.P. Designs for clinical cancer research. Discussion II: Critical definitions/criteria for evaluation. Cancer Treat. Rep. 64: 469-471, 1980.

Byar, D.P.: Why data bases should not replace randomized clinical trials. Biometrics 36: 337-342, 1980.

Byar, D.P. and Green, S.B.: The choice of treatment for cancer patients based on covariate information: Application to prostate cancer. Bulletin du Cancer 67: 477-490, 1980.

Day, N.E., Byar, D.P., and Green, S.B.: Overadjustment in case-control studies. Am. J. Epidemiol. 112: 696-706, 1980.

Gail, M.H.: Competing risks. In Kotz, S., and Johnson, N.L. (Eds.): Encyclopedia of Statistical Sciences, Vol. 1, New York, John Wiley & Sons, in press.

Gail, M.H.: Evaluating serial cancer marker studies in patients at risk of recurrent disease. Biometrics 37: 67-78, 1981.

Gail, M.H., Lubin, J.H. and Rubinstein, L.V.: Likelihood calculations for matched case-control studies and survival studies with tied death times. Biometrika, in press.

Gail, M.H., Santner, T.J., and Brown, C.C.: An analysis of comparative carcinogenesis experiments based on multiple times to tumor. Biometrics 36: 255-266, 1980.

Green, S.B.: Randomized clinical trials: Design and analysis. Seminars in Oncology, in press, 1981.

Muenz, L. and Sizaret, P.: On an international study of the alpha-fetoprotein standard and statistical methodology for radioimmunoassays. In Weitzel, H.K. and Schneider, J. (Eds.): Alpha-Fetoprotein in Clinical Medicine. Stuttgart, Hannover, Germany, George Thieme Publishers, 1979, pp. 157-168.

Rubinstein, L.V., Gail, M.H., and Santner, T.J.: Planning the duration of a comparative clinical trial with loss to follow-up and a period of continued observation. Journal of Chronic Diseases, in press 1981.

Slud, E.V., and Kedem, B.: On goodness of fit of time series models: An application of higher order crossings. Biometrika, in press, 1981.

Slud, E.V.: Loss of information from transforming stationary time series. To appear In: Anderson, O.D. and Perryman, M.R. (Eds.), Proceedings of International Time Series Meeting, Houston, 1980, in press.

Wei, L.J.: Asymptotic conservativeness and efficiency of Kruskal-Wallis test for K dependent samples. Journal of the American Statistical Association, in press.

Wei, L.J.: Estimation of location difference for fragmentary samples. Biometrika, in press.

Wei, L.J.: The Friedman's Urn. In John, N.L., and Kotz, S. (Eds.), The Encyclopedia of Statistical Sciences, Vol. III, New York, John Wiley, in press.

Wei, L.J.: The Gilbert's Test. In: Johnson, N.L., and Kotz, S. (Eds.), The Encyclopedia of Statistical Sciences, Vol. II, New York, John Wiley, in press.

Wei, L.J.: Interval estimation on location difference with missing observations. Biometrika, in press, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CP 04414-06 B

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Relation of Premorbid Psychological Factors to Cancer Incidence

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Bernard H. Fox Manager, Social Science BB NCI

COOPERATING UNITS (if any)
Johns Hopkins Univ., Baltimore, MD.
Univ. of Lund, Lund, Sweden
Stanford Univ., Palo Alto, CA.
Rush-Presbyterian-St. Luke's Med. Center, Chicago, IL.
No. California Cancer Program, San Francisco, CA.

LAB/BRANCH
Biometry Branch, Field Studies and Statistics Program

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD. 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.54	.50	.04

CHECK APPROPRIATE BOX(ES)

XX (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS XX (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The association of premorbid psychological factors--stress, personality, social environment--and cancer incidence and cancer mortality has been proposed and argued about. The possibility of such an association continues to be examined. Prospective studies on various cohorts are being pursued: a) an employee cohort showed no relationship of repression test scores to cancer incidence or mortality over the following 20 years, and verified, in later cancer incidence cases, a previously found positive relationship of depression scores to later cancer mortality; b) a college cohort showed a relationship between men's scores on a set of 15 psychological items and colon cancer mortality over the following 35 years; c) follow-up of an adult Swedish county cohort for a further 20 years after an initial 10-year follow-up indicated an existing but reduced relationship of cancer mortality and specific psychological test scores in the 2nd 10-year follow-up; and d) follow-up of a cohort of men with Type A and B personalities over 10 years showed a positive but non-significant risk for Type A. The theory of the relationship of psychological factors to cancer initiation and progress has been clarified somewhat and is being investigated further.

Project Description

Objectives:

To determine if premorbid psychosocial factors--stress, personality, social environment--are associated with initiation or rate of progress of cancer.

Methods Employed:

In each of four studies, a presumed cancer-free cohort was available, with certain psychological tests given at the beginning of the study period: a) in 2,000+ 40-55 year-old male employees of Western Electric in Chicago in 1958, their cancer incidence and mortality was followed through 1977; b) in about 39,000 University of Pennsylvania (male and female) and Harvard (male) students in the 1930's, cancer mortality and incidence has been followed through 1976; c) in 1,200+ adults in two Swedish counties in 1947, cancer mortality has been followed through 1967 and will be followed through 1972; and d) about 3,000 middle-aged men in the original Type A cohort from northern California, in 1961, have been followed through 1970, and will be followed through 1980.

Major Findings:

A) In the Western Electric cohort, although a relationship of depression to later cancer mortality and incidence was demonstrated, repression was not so related. However, an analysis of diet showed a marked inverse relationship between incidence of lung cancer and level of carotene ingestion. B) In the college group, men with high scores on a group of psychological items had a significantly elevated risk of dying from colon cancer, but not so for living cancer patients. No relationship was found for any other type of cancer, nor for any other set of items in the questionnaire. C) Preliminary analysis of data from 1957-1967 in the Swedish cohort showed some relationship of scores on the Sjobring test to later cancer mortality, but weaker than that displayed in 1947-1957. The evidence for the original hypothesis of a Sjobring-mortality relationship is not holding up strongly. D) Preliminary data from the Type A cohort show that the relative risk among Type A personality males of dying of cancer in the 1960's decade was 1.36, which is not significant. Only 41 cases appeared, however. Data from the 1970's are expected to show about 80 more deaths, and a more trustworthy relative risk figure.

Significance to Biomedical Research and the Program of the Institute:

Repeatedly NCI has received questions from professionals and the public about the relation among psychological factors, stress and cancer. These questions were based on a long history of belief, speculation and purported evidence that stress and certain pschological factors increase cancer risk. Evidence on humans, though voluminous, is not persuasive, and in some cases, contradictory. Evidence on animals is stronger, and shows both reduced and increased susceptibility to tumors. Most of the human studies have not taken into account certain factors having an important impact on outcomes. Studies

with improved design are, therefore, much needed, and these projects fulfill that need, in part. A positive answer would give impetus to studies on specific psychological factors or stress and the humoral environment associated with increased cancer risk. A negative answer would tend to shift the balance of research effort away from the psychic components of environmental carcinogenesis, whether internal or external to the body.

Proposed Course of Project:

Of the above four studies, the first two will further pursue the relationship of psychological factors and cancer. The Swedish study will be pursued through 1972, and a substantial increase in cancer cases is expected. The mortality study on Type A men will be continued with 1970's data, and also with respect to incidence, the latter mostly funded by the NHLBI. NCI is expected to fund a small portion of the study, specifically as it relates to psychological factors. Covariates will be analyzed. They are expected to be complete within a year, although the incidence data may take longer to get.

Publications:

Fox, B.H. A psychological measure as a predictor in cancer. In Cohen, J., Cullen, J.W. and Martin, L.R. (Eds.): Psychosocial Aspects of Cancer. New York, Raven Press, 1981. In press.

Fox, B.H. Behavioral issues in cancer. In Weiss, S.M., Herd, J.A. and Fox, B.H. (Eds.): Perspectives on Behavioral Medicine. New York, Academic Press, 1981. pp. 101-133.

Fox, B.H. Endogenous psychosocial factors in cross-national cancer incidence. In Eiser, J.R. (Ed.): Social Psychology and Behavioral Medicine. London, Wiley. In press.

Fox, B.H. Psychosocial factors and the immune system in human cancer. In Ader, R. (Ed.): Psychoneuroimmunology. New York, Academic Press, 1981. pp. 103-157.

Weiss, S.M., Herd, J.A. and Fox, B.H. (Eds.): Perspectives on Behavioral Medicine. New York, Academic Press, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04475-05 B

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

National Nonmelanoma Skin Cancer Incidence and Epidemiology Studies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I. :	Joseph Scotto	Director, Health Services Director	BB	NCI
Other:	Thomas R. Fears	Mathematical Statistician	BB	NCI

COOPERATING UNITS (if any)
Interfederal Committee on Stratospheric Ozone Protection (ICSOP)
Environmental Protection Agency
National Oceanic and Atmospheric Administration

LAB/BRANCH
Biometry Branch, Field Studies and Statistics Program

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INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	2	

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project, a collaborative effort by the NCI, EPA and other federal agencies, was initiated to provide more data relative to skin cancer and harmful solar ultraviolet. An urgent need for these data has existed since recent scientific reports have warned about the decomposition of stratospheric ozone by nitogren oxides and chlorofluoromethanes (CFM's). Federal regulatory agencies have already recommended bans on the use of aerosol spray cans which use CFM's as propellants. In the near future, there will be critical reviews of all pertinent information by government as well as individual interests concerned with the threat of increased human skin cancer due to excess amounts of UV-B reaching the earth's surface, as its protective ozone shield is depleted. As mandated by Public Law 95-95 (amendment to the Clear Air Act of 1977), the NCI is continuing its investigations in this area. This will provide more reliable national estimates of the degree of morbidity from nonmelanoma skin cancer as well as epidemiologic data on host and environmental factors, other than solar ultraviolet, associated with increased risk. New leads from 10 surveys just being completed will be investigated.

PHS-6040
(Rev. 2-81)

Project Description

Objectives:

The major objectives of this study are to provide epidemiologic data relative to the etiology of nonmelanoma skin cancer, and to evaluate the potential human health effects of harmful solar ultraviolet (UV-B, i.e., wavelengths between 290 nm and 320 nm). In particular, (1) to provide information necessary to ascertain the human health effects of UV radiation resulting from the anticipated ozone depletion in our biosphere; (2) to provide supportive basic data to reduce the degree of uncertainty in dose-response estimators; (3) to provide specific data on populations suspected to be at high or low risk of skin cancer; (4) to provide an estimate of the proportion of skin cancer in the community relative to other cancers; (5) to identify local factors in the community that may contribute to the risk of skin cancer; (6) to provide basic data in support of anticipated needed preventive care programs in this community; (7) to provide basic epidemiologic data to elucidate the multifactorial etiology of skin cancer; and (8) to estimate trends in skin cancer morbidity.

Methods Employed:

Ten population-based registries were developed in various geographic locations of the contiguous United States where latitudes ranged from 47°N to 30°N. Incidence information on newly diagnosed nonmelanoma skin cancer (basal cell and squamous cell carcinoma) was collected in accordance with protocols developed by NCI. Surveys are conducted in a uniform way so as to provide data bases comparable with earlier surveys conducted by NCI in 1971-72. Relevant environmental epidemiologic data and other information on host factors are also being collected using case/control methods on a sampling basis. Interviews are conducted via telephone communication. In addition UV-B measuring devices, i.e., Robertson-Berger meters, were installed and maintained at these locations in collaboration with the National Oceanic and Atmospheric Administration (NOAA). This federal agency will provide data from these locations to the NCI as they come available. Eight registries are now completed: Seattle, Minneapolis, Detroit, Utah, San Francisco, New Mexico, Atlanta, and New Orleans. In the southwest region, San Diego, California was recently completed and the analytic data files for incidence and other epidemiologic information are in their final stages of completion. In the northeast region, a study in New Hampshire-Vermont has been successfully implemented, and new incidence data are in the final stages of completion. We anticipate the interview phase for New Hampshire-Vermont to be implemented by late summer, 1981.

Major Findings:

A preliminary report of the basic incidence data has been published. The new data suggest that among Caucasians in the United States the annual incidence of nonmelanoma skin cancer may be in the neighborhood of 400,000. This annual amount is about half that observed for all other cancers combined. While no significant increases in UV-B or decreases in

stratospheric ozone depletion may be detected in the short time since these measurements were being made by NOAA, there appears to be an increase in the incidence of basal cell carcinomas of the skin among Caucasians in Minneapolis-St. Paul and San Francisco-Oakland. The increase, 15 to 20 percent over a six year period, was not noted for squamous cell carcinomas for all anatomical sites combined. Over 80 percent of the basal cell tumors arise in the face, head or neck; but the predilection for these exposed sites is somewhat less for squamous cell carcinomas. The risk for males is about two-fold greater than that for females, with greater excesses for males among squamous cell carcinoma. The north-south incidence gradient appears to be steeper for squamous cell carcinoma than for basal cell carcinoma. The amount of skin cancer among the non-Caucasian races was quite negligible. Among whites, those who do not burn and could tan easily were less likely to develop skin cancers. Those with histories of treatment for skin diseases, such as ionizing radiation exposure for acne or moles, or with perhaps increased UV radiation exposure for psoriasis, appear to be at greater risk. Fair skinned individuals of Irish or Scottish ancestry were, as expected, more susceptible to skin cancer. It was surprising, however, to find that those of Scandinavian ancestry, who are usually perceived as being very fair skinned, were apparently not at greater risk than other Caucasians of either German or English descent.

Using eight new locations and two old locations in our analyses of the dose-response relationship of UV-B and skin cancer, we have substantially improved the degree of reliability in our estimates. Mathematical models applied to the new data indicate that a one percent increase in UV-B may result in a somewhat less than 2 percent increase in skin cancer. This implies that stratospheric ozone reductions of one percent may eventually result in a 4 percent increase in skin cancer. With large decreases in stratospheric ozone (over 10%) the subsequent increases in skin cancer may be even greater than four-fold.

Significance to Biomedical Research and the Program of the Institute:

These data when combined with those from earlier surveys will provide a basis for evaluating the potentially harmful health effects of ozone depletion in our biosphere. They will also provide new leads on the relative importance of host factors and environmental factors other than UV-B which may contribute to increased risk for this disease. Results from these data help the EPA and other regulatory agencies establish guidelines for use of man-made products, such as chlorofluoromethane propellants in aerosol spray cans and refrigerants in air conditioners, which may effect human health. The new data provide current estimates of the degree of morbidity from skin cancer in various parts of the United States, and elucidate the need for cancer prevention programs.

Proposed Course:

Intensified analyses of the newly developed data bases will continue. In addition to the basic incidence reporting, new leads from the current surveys (e.g., excessive radiation exposure, high risk occupation/industry

groups, skin conditions such as psoriasis, residence mobility such as movements from northern to southern "sun spots," etc.) are being pursued. Because of the the apparent difference in incidence patterns for squamous cell carcinoma, more detailed analyses and studies will be pursued which will isolate cell type and etiology. In our current interview study in New Hampshire-Vermont, we will attempt to interview as many squamous cell carcinoma patients as possible.

We envision doing field studies utilizing newly developed personal UV-B dosimeters. At present we know how much UV-B may reach the surface of the earth, but we are severely lacking in information on the relative amounts which may reach exposed surfaces of the human skin. Our plans also include conducting new skin melanoma case/control studies into the major framework of this project. The etiology of skin melanoma also reflects an association of increased risk at locations of high insolation, but the relationship is complicated by the apparent contradiction of anatomical site distributions of malignant melanoma lesions. The short-term funding for this project will have been used by September 1981. These new initiatives cannot be implemented without a new funding commitment, and it appears that other federal agencies (such as EPA) may not provide any transfer funds as they have in the past.

Publications:

Scotto, J. and Fears, T.R.: Skin Cancer Epidemiology: Research Needs. In Proceedings of the International Conference on Ultraviolet Carcinogenesis. Natl. Cancer Inst. Monogr. 50: 169-177, 1979.

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Nam, J. and Scotto, J.: Study of Seasonality, The Authors Reply. American J. of Epidemiology 113: 481, 1981.

Scotto, J., Fears, T.R., Lisiecki, E., and Radosevich, S.: Incidence of Non-melanoma Skin Cancer in the United States, 1977-78: Preliminary Report. NIH Publ. No. 80-2154, Washington, D.C., U.S. Government Printing Office, 1980, pp. 1-24.

Scotto, J. and Fears, T.R.: Skin Cancer in the United States. In Schneiderman, M.A. and Levin, D.L. (Eds.): Monogr. Cancer in the USA and USSR, National Cancer Institute, NIH, Publ. No. 80-2044, 1980, pp. 129-136.

Scotto, J., Fears, T.R., and Fraumeni, J.F., Jr.: Solar Radiation. In Schottenfeld, D. and Fraumeni, J.F., Jr. (Eds.): Cancer Epidemiology and Prevention. Chapter 14. Philadelphia, W.B. Saunders and Company. In Press.

Scotto, J. and Fraumeni, J.F., Jr.: Skin (other than Melanoma). In Schottenfeld, D. and Fraumeni, J.F., Jr. (Eds.): Cancer Epidemiology and Prevention. Chapter 60. Philadelphia, W.B. Saunders and Company. In Press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04482-05 B

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Psychological and Other Predictors of Remission Time After Surgery In Clinical State II Melanoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Bernard H. Fox	Manager, Social Science	BB NCI
	Daniel Van Kammen	Unit Chief, Section on Neuropsychopharmacology	BP NIMH

COOPERATING UNITS (if any) This is a sub-project of a broader one, Project Z01-CB-05052-I, Psychological Factors in Prognosis of Melanoma, whose PI was G. Nicholas Rogentine, Jr. until 11/15/78, when he left, and his project was discontinued. Immunology Branch, NCI, continues to cooperate by providing data.

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Biometry Branch, Field Studies and Statistics Program

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NCI, NIH Bethesda, Md. 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.11	.1	.01

CHECK APPROPRIATE BOX(ES)

XX (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS XX (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In this study, an attempt has been made to assess the ability of certain psychological measures to discriminate those stage II melanoma patients who relapsed within one year after axillary node surgery, and to test their ability to predict such relapse in an independent sample. From an initial sample of 31 cases, the successful discriminators, number of nodes and a psychological variable--subjective effect of having melanoma--held up as predictors in an independent sample of 33 cases with about equal success. Preliminary analyses of the 1-year predictors of relapse suggested no such capability for either 2-year overall relapse or 2-year overall mortality, but separate analyses of the two samples showed a significant difference between them, results being in opposite directions. Mortality for all 64 cases in the sample is being ascertained for 2 years following testing, and an attempt will be made to obtain 3-year mortality data.

PHS-6040
(Rev. 2-81)

Project Description

Objectives:

To test hypotheses that certain psychological measures will predict relapse status and mortality, after controlling for known physical discriminators.

Methods Employeed:

Thirty-eight psychological measures (MMPI scales, SCL-90 psychiatric symptoms scale, and several other measures) were given to 31 stage II melanoma patients a few weeks after surgical removal of axillary lymph nodes. A number of physical measures were also taken in these patients. Ability of each of these to discriminate those who did or did not relapse before one year was determined, both by means of a clinical algorithm and by discriminant analysis. After identifying discriminators, their ability to predict was tested in an independent sample of 33 patients. Further analysis of relapse at 2 years and mortality at 2 years was begun and is being continued, and mortality experience by 3 years is being investigated.

Major Findings:

A) In the initial sample, aside from the MMPI, only the number of nodes and the psychological variable, subjective effect of melanoma on life style, discriminated 1-year relapsers successfully. When tested separately on a new sample of 33 patients, number of nodes and subjective effect of melanoma on life style also discriminated relapsers successfully. B) Some MMPI scales discriminated on the first sample ($p < .20$) and some did on the 2nd, but none did on both. Certain scales correlated with nodes and subjective effect--an interesting, but not important, result. C) No physical variables discriminated relapsers from non-relapsers other than number of nodes. A preliminary analysis of the 1-year predictors of relapse showed no such capability for either 2-year relapse overall or 2-year mortality overall, but separate analyses of the two samples showed results in opposite directions, suggesting sample differences or strong variability. The preliminary indications for relapse at 2 years have been confirmed, and for mortality they are almost fully confirmed.

Significance to Biomedical Research and the Program of the Institute:

If psychological variables are found to have independent weight in predicting remission time, their relationship with some physical attributes should be sought to establish why they are predictors. The most important outcome would entail a cause-effect relationship of psychic state and such physical variables, thus permitting change and possible extension of remission. Even without such a relationship, a predictive capability of psychological measures would be valuable to the clinician, and under some conditions, perhaps to the family and the patient. These results offer only interesting hypotheses about personality in the acute surgical case and shortly thereafter, but fail to support psychological relationships with relapse or death later in the disease.

Proposed Course:

The findings above (C. Major Findings) will be pursued to obtain full ascertainment of two year mortality. An attempt will be made to ascertain 3-year relapse and mortality.

Publications:

None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CP 05158-01 B

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)
Morbidity Among Long-Term Survivors of Childhood Cancer and Their Offspring

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PIs:	Max H. Myers, Ph.D., Chief, Biometric Research and Analytic Studies Section		BB NCI
	John J. Mulvihill, M.D., Chief, Clinical Genetics Section		CEB NCI
Other:	Sandra C. Abbott	Statistician (Health)	BB NCI
	Roger R. Connelly	Statistician (Health)	BB NCI
	Margot R. Hanson	Statistician (Health)	CEB NCI

COOPERATING UNITS (if any) University of Iowa, University of Kansas, University of Texas System Cancer Center, Yale University School of Medicine, California State Department of Health, Westat Inc., Geomet Inc.

LAB/BRANCH
Biometry and Clinical Epidemiology Branches, Field Studies and Statistics Program

SECTION
Biometric Research and Analytic Studies and Clinical Genetics Section

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.4	1.4	1

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Basic data tapes providing descriptive information on demographic characteristics and cancer diagnoses have been submitted to NCI. Medical record abstracting and questionnaire administration has begun. Some elements of the data management system are operational (logging in of submitted questionnaires and abstracts) and work is underway on development of procedures for data entry, editing, updating and analysis.

PHS-6040
(Rev. 2-81)

Project Description

Objectives:

To determine the long-term effects of cancer and its treatment on patients who reached adulthood after having cancer during childhood years.

To assess any adverse effects that might have carried over to offspring of childhood cancer survivors.

To test genetic theories of tumor etiology.

Methods Employed:

A total of 2,854 survivors of childhood cancer were identified at the 5 cooperating centers. Up to 2 sibling controls are being sought for each case. Each subject (case or control) is being asked to provide information gathered by means of an in-person interview regarding the following:

- fertility problems
- pregnancy wastage
- congenital anomalies in cases and offspring
- second primary neoplasms in cases
- cancers in offspring
- psychosocial morbidity

Analytical techniques will include methods for risk estimation for matched case-control triads and methods based upon stratification.

Major Findings:

This project has just entered the data collection phase so there are no findings to report.

Significance to Biomedical Research and the Program of the Institute:

Prior to this study there has been very little information concerning possible residual effects of cancer and its treatment on surviving childhood patients and their offspring. This study is large enough to identify important sequel events for children treated for cancer during a period prior to the most recent advances in combination chemotherapy and multi-modal therapies.

Proposed Course:

Data collection is expected to be completed during the spring of 1982. Preliminary examination of the data will be done as sufficient numbers of cases and controls are entered into the computer file. Published results are expected by late 1982 or early 1983.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 05176-01 B

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Statistical Methodology - Consultation and Research

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Charles C. Brown, Ph.D., Mathematical Statistician BB NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Biometry Branch, Field Studies and Statistics Program

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	1.0	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to collaborate with NCI and other federal researchers on statistical problems related to many areas of cancer research, and to conduct research on the development of statistical methodology which is particularly applicable to the analysis of data from experimental and epidemiological studies of cancer. Particular subjects of interest are methods of quantitative risk assessment, methodology for analyzing survival curves and proportions, and the analysis of epidemiologic studies, based on the multistage theory of carcinogenesis.

Project Description

Objectives:

The principal objectives are (1) to consult and collaborate with NCI and other federal agency researchers on statistical problems related to cancer research, and (2) to conduct research on the development of statistical methodology which is applicable to the design, analysis and interpretation of experimental and epidemiologic studies of cancer.

Methods Employed:

The methods employed are the modern theories of mathematical statistics, probability, applied mathematics, and epidemiology. The development of computer programs is often used in the application of these methods.

Major Findings:

The research conducted under this project covers a wide spectrum of topics which are summarized below.

A paper presenting an examination of the validity of commonly used interval estimators for the odds ratio in a 2x2 table was completed. This study shows that Cornfield's method of interval estimation is a valid method whereas Woolf's method is often substantially invalid for small sample sizes and Miettinen's method has an inherent bias which increases as the true odds ratio deviates from unity.

A paper presenting an extension of a method for testing the goodness-of-fit for logistic models based on score statistics was completed. This method does not suffer from some of the weaknesses of other methodologies and has good power against certain members of a general class of alternative models.

A methodology accepted for publication was developed with Dr. Sylvan B. Green, of the Clinical and Diagnostic Trials Section, for the computation of sample sizes for comparing two Poisson outcomes. This extends the work of Dr. Mitchell H. Gail to the case of unequal sample sizes and is applicable to epidemiologic and experimental studies of rare disease occurrence.

A methodology was developed for the comparison of two or more relative survival curves (i.e., survival adjusted for 'expected' survival based on age, sex, and race). This methodology was shown to be 'optimal' in a certain sense, and for the first time allows a statistical comparison of relative survival rather than observed survival.

A methodology was developed with Dr. Thomas R. Fears for the exact calculation of an overall false positive rate when making multiple 2x2 comparisons between treated and control animals in a carcinogenesis bioassay. This work is planned to be extended to the situation in which more complex statistical comparisons would be made (e.g., tests adjusted for time of tumor occurrence or discovery, and tests for trend with increasing dose).

New methodologies for the analysis and interpretation of epidemiologic studies of occupational carcinogenesis based on the multistage theory of carcinogenesis are being developed in collaboration with Dr. Kenneth C. Chu of the Technical Resources Branch. These methodologies have been applied to a cohort of workers exposed to arsenic with the tentative conclusions that arsenic primarily appears to exert its carcinogenic influence at a late stage in the carcinogenic process, but also may affect an early stage. This work is planned to be extended to a recently completed extension of the follow-up of this cohort.

An analysis of the increase in cancer incidence among the Japanese exposed to the A-bomb radiation is continuing. These data are being examined and interpreted based on the multistage theory of carcinogenesis. In collaboration with Dr. H. Kato, of the Radiation Effects Research Foundation (RERF), and with Dr. David G. Hoel and Dr. William J. Schull, while visiting researchers at the RERF, work is continuing on Report 9 of the Life Span Study, 'Part II: Mortality from Causes other than Cancer 1950-1978'. This report shows that, after correcting for the competing causes of death due to cancer, other mortality shows little, if any, relation to the level of radiation exposure.

Collaboration with John W. Horm is continuing on the development of a system for the monitoring of cancer incidence data being collected by the SEER Program.

Consultation has begun with researchers involved in the development of a record linkage system for the management of SEER data systems. This consultation is planned to continue through the implementation of the system for the various SEER registries.

In collaboration with John W. Horm and Lynn G. Ries, a study has been started of the differences in stage of disease and subsequent survival of the large increase in the incidence of diagnosed breast cancer in women which occurred shortly after the cancers diagnosed in Mrs. Ford and Mrs. Rockefeller.

Consultation is continuing with other researchers in the Biometry Branch, Rochelle Curtis and Dr. Earl S. Pollack, on the application of the logistic model to the analysis of breast cancer and G.I. cancer incidence.

In the area of the assessment of human carcinogenic risk based upon experimental animal studies, consultation and collaboration with researchers from the federal regulatory agencies is continuing. The primary work in this area over this time period has been (1) in collaboration with Daniel Krewski of the Health Protection Branch, Health & Welfare Canada, preparation of a bibliography of reports on carcinogenic risk assessment to be published in two journals, one statistical in nature and one toxicological; (2) in collaboration with other researchers in a working group sponsored by the EPA, preparation of a report, 'Considerations in Evaluating Risk to Male Reproduction;' (3) preparation of a chapter in the forthcoming book, 'Principles for the Evaluation of Toxic Hazards to Human Health' edited by Dr. Robert G. Tardiff and Dr. Joseph V. Rodricks, and (4) general consulting

with regulatory agency researchers in the area of statistical methodology and data interpretation.

Significance to Biomedical Research and the Program of the Institute:

The relationship of statistical theory to experimental research and data analysis is an important aspect of carcinogenic research. The research objectives of the Institute and other workers in cancer research are promoted by the continued work on the development of new statistical methodologies such as those presented above. The opportunity for conducting fundamental research on mathematical statistics is essential to achieve professional peer recognition. More importantly, the possibility of doing such research is necessary to carry out consulting activities at the highest professional level.

Proposed Course:

Much of the work described in the major findings will be continued. Other research and consulting projects, not described above, are also likely to be initiated. The current balance between research and consultation, theory and application, is anticipated to continue unchanged.

Publications:

Gail, M.N., Santner, T.J. and Brown, C.C.: An analysis of comparative carcinogenesis experiments based on multiple times to tumor. Biometrics 36: 255-266, 1980.

Brown, C.C.: The validity of approximation methods for interval estimation of the odds ratio. Amer. J. Epidem. 113: 474-480, 1981.

Wahrendorf, J., Zentgraf, R. and Brown, C.C.: Optimal designs for the analysis of interactive effects of two carcinogens or other toxicants. Biometrics 37: 45-54, 1981.

Wahrendorf, J. and Brown, C.C.: Bootstrapping a basic inequality in the analysis of joint action of two drugs. Biometrics 36: 653-657, 1980.

Thompson, H.J., Becci, P.J., Brown, C.C. and Moon, R.C.: Effect of the duration of Retinal Acetate feeding on inhibition of 1-Methyl-1-nitrosourea induced mammary carcinogenesis in the rat. Cancer Research 39: 3977-3980, 1980.

Krewski, D. and Brown, C.: Carcinogenic risk assessment: A guide to the iterature. Biometrics (in press).

Krewski, D. and Brown, C.: Carcinogenic risk assessment. A guide to the literature. Toxic Substances Journal (in press).

Brown, C.C. and Fears, T.R.: Exact significance levels for multiple binomial testing with application to carcinogenicity screens. Biometrics (in press).

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Commonwealth of Puerto Rico (NO1-CP-43386)	SEER Program	"
Fred Hutchinson Cancer Research Center, Seattle (NO1-CP-61059)	SEER Program	"
Research Corp. of the Univ. of Hawaii (NO1-CP-53521)	SEER Program	"
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CONTRACT NARRATIVE
DEMOGRAPHIC ANALYSIS SECTION

CALIFORNIA STATE DEPARTMENT OF HEALTH (NO1-CP-81018)
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COMMONWEALTH OF PUERTO RICO (NO1-CP-43386)
CONNECTICUT STATE DEPARTMENT OF HEALTH (NO1-CP-61002)
EMORY UNIVERSITY (NO1-CP-61027)
FRED HUTCHINSON CANCER RESEARCH CENTER, SEATTLE (NO1-CP-61059)
RESEARCH CORP. OF THE UNIVERSITY OF HAWAII (NO1-CP-53511)
UNIVERSITY OF IOWA (NO1-CP-43200)
MICHIGAN CANCER FOUNDATION (NO1-CP-61028)
UNIVERSITY OF NEW MEXICO (NO1-CP-33344)
UNIVERSITY OF UTAH (NO1-CP-43382)
YALE UNIVERSITY (NO1-CP-33235)

Title: Surveillance, Epidemiology & End Results (SEER) Program

Project Officer (NCI): John L. Young, Jr., Dr.P.H.

Objectives: To obtain and analyze data on cancer morbidity, treatment, extent of disease, and patient survival from population-based registries; to identify areas for epidemiologic investigations; to initiate preliminary investigations needed to develop epidemiologic study protocols; and to promote specialty training in tumor registry methodology.

Methods Employed: The National Cancer Institute is sponsoring a collaborative program for Cancer Surveillance, Epidemiology and End Results (SEER). Participants in this program are all population-based registries, covering entire states or geographically specified areas, such as Standard Metropolitan Statistical Areas. The Demographic Analysis Section has professional and technical responsibility for supervising these contracts. Basic information is collected on all patients treated for cancer in the area, area residents treated outside the area, and anyone dying in the area whose death certificate mentions cancer--or area residents dying outside the area, whose death certificate mentions cancer. Periodic follow-up information is obtained on the vital status of all those patients in the registries. Data are submitted semi-annually to NCI on magnetic tape according to a specified format. These data are available for analyses as well as for providing an extensive resource for special studies.

Core epidemiologic staff are present in most of the SEER Programs. These staff members review the morbidity data to identify epidemiologic research leads that should be investigated. These staff then develop study protocols and field test the study schedules. Funding for such proposals are either through other contract or grant support or financial assistance from other sources.

Significance to Biomedical Research and the Program of the Institute: Information on incidence, survival and mortality from a cross section of the U.S. population is required on a continuing basis so that the nature and magnitude of the cancer problem, including changes over time, can be determined. Through close scrutiny of variation in cancer incidence and survival with respect to geographic and demographic characteristics of the population and differential changes over time, specific etiologic hypotheses will emerge which, when tested

via special study mechanisms, should lead to the identification of controllable risk factors. Such research is an integral part of the ultimate goal of the national program; i.e., to reduce the occurrence of and mortality due to cancer. Continued recording of cases will also provide a data base that could be useful in the evaluation of cancer control activities. These data could provide the base-line against which changes resulting from specified cancer control programs could be measured to determine the effectiveness of these programs in reaching their stated goals. A system of record linkage between the cancer registry and employees of specific industries or specific types of occupations would provide early warning of possible occupational carcinogenicity.

Major Findings:

Data for cases diagnosed between January 1, 1973 and December 31, 1979 were submitted to NCI in December 1980. One participant submitted data only for 1974-1978; one submitted data only for 1975-1978. While it is felt that all areas have complete reporting for 1978, data for 1979 appear to be incomplete. In addition, survival data for patients diagnosed between January 1, 1973 and December 31, 1978 are available through at least December 31, 1979. There was considerable variation among the participants with respect to the percent of patients not known to be dead who were actually followed into calendar year 1979 or 1980. Three of the ten participants have rates which are unacceptably low and major efforts to improve this deficiency are underway.

Incidence and mortality data for 1973-1977 for the eleven participants included in the program at that time have been published in NCI Monograph 57. A synopsis of the major findings of those data were as follows:

The average annual age-adjusted incidence rate for all forms of malignant neoplasms for all races and both sexes combined for all SEER areas except Puerto Rico was 331.5, whereas the rate for Puerto Rico was 200.4. Among the other areas, rates ranged from a low of 279.8 in Utah to a high of 363.5 in San Francisco-Oakland.

The average annual rate of carcinoma in situ for all SEER areas excluding Puerto Rico was 26.7; the rate for Puerto Rico was 16.9. Among the other areas, these rates ranged from a low of 19.2 in Utah to a high of 41.8 in Atlanta.

The most common primary sites of cancer were colon, rectum, breast, and lung with age-adjusted rates of 48.5, 46.7, and 46.7, respectively. In fact, these 3 sites accounted for 42.7% of all malignant cancers. Seventy-eight percent of the in situ lesions occurred in the female genital system.

The average annual mortality rate due to malignant neoplasms for all SEER areas, excluding Puerto Rico, was 168.5 compared with a rate of 166.5 for the total United States. The comparable rate for Puerto Rico was 127.0. Rates ranged from a low of 123.6 in Utah to a high of 201.1 in New Orleans. The mortality rate for Puerto Rico was slightly higher than that for Utah, even though the incidence rate was considerably lower (200.4 vs. 279.8). This difference reflects the higher proportion among Puerto Rican patients with cancer at primary sites for which survival is poor (esophagus, stomach, liver) than in patients in Utah. Similarly, the high mortality rate in New Orleans is reflective of the high incidence of lung cancer in that area.

Of all cancer deaths, lung cancers accounted for 21.7%; colorectal, 13.6%; and breast cancers, 9.2%. These 3 sites accounted for over 40% of cancer cases and deaths, although proportionately more deaths were attributable to lung cancer.

Considerable variation is noted in both incidence of and mortality from cancer among the various racial and ethnic groups for which data are available. The following list shows the variation in average annual age-adjusted incidence rates for all sites combined among the races for each sex:

Males

Hawaiian, Hawaii (465.0)
Black, all areas (454.3)
White, all areas (371.6)
Japanese, Hawaii (327.6)
Chinese, San Francisco-Oakland (325.6)
Chinese, Hawaii (262.9)
Filipino, Hawaii (249.5)
Hispanic, New Mexico (229.5)
Hispanic, Puerto Rico (229.2)
Japanese, San Francisco-Oakland (222.0)
American Indian, New Mexico (178.4)

Females

Hawaiian, Hawaii (408.5)
White, all areas (301.2)
Black, all areas (288.7)
Chinese, San Francisco-Oakland (283.6)
Chinese, Hawaii (263.0)
Hispanic, New Mexico (237.1)
Japanese, San Francisco-Oakland (224.0)
Japanese, Hawaii (220.9)
American Indian, New Mexico (191.6)
Filipino, Hawaii (191.5)
Hispanic, Puerto Rico (173.6)

In addition to data for 1973-77, data for 1978 and 1979 are also available. Since New Orleans data are not available past 1977, comparison of numbers of cases and age-adjusted rates for 1978 and 1979 with those for 1973-1977 must be interpreted in that light. Currently, data exist on 70,082 and 70,327 malignant cases for 1978 and 1979, respectively, for the nine geographic areas, excluding Puerto Rico, and 4,980 and 5,034 malignant cases for 1978 and 1979, respectively, from Puerto Rico.

Because of difficulties in estimating the population of the various minority and ethnic groups, incidence rates for only whites and blacks have been produced for 1978 and 1979.

Significance to Biomedical Research and the Program of the Institute:

Proposed Course:

Intensive effort has been expended to assure that the data being submitted to NCI are of the quality and completeness required for an effective cancer surveillance

network. Incidence data are now of high quality. Effort will be expended to improve the quality of data collected for patient survival so that the percentage of alive patients under active surveillance will reach at least 85%. The quality control staff in cooperation with the training staff of the University of California Medical School in San Francisco will continue to conduct a program of quality control visits, as well as national and regional workshops, and intensive formal training programs.

To assist in tumor registry training programs and to provide guidance to SEER Program Participants and others concerned with cancer registration, a number of publications have been developed and distributed. These include manuals that set out clear guidelines for collecting and coding the data to be submitted to NCI, as well as books that can be used for on-the-job training of personnel responsible for carrying out the functions required for data extraction and coding. The demand for these publications has been large and mailings have been made to a variety of medical personnel and facilities. A new book documenting antineoplastic drugs is ready for distribution. Additional books on statistical and epidemiological methodology and computer assistance for the tumor registrar are being developed. Earlier books on anatomy and extent of disease are being revised.

Incidence and mortality data covering the first five years of the Program are published in the NCI Monograph series. The volume contains over 1,000 pages and includes data classified by race, sex, age, geographic area, primary site and histologic type of cancer. A companion monograph containing data on cancer patient survival classified by race, sex, age, and residence of the patient and by site, type, extent of disease and treatment will be developed.

	Date Initiated	FY 81 Negotiated
California State Department of Health (NO1-CP-81018)	1962	$1,817,660
California, University of, San Francisco (NO1-CP-11004)	1974	252,984
Commonwealth of Puerto Rico (NO1-CP-43386)	1968	164,665
Connecticut State Department of Health (NO1-CP-61002)	1963	780,100
Emory University (NO1-CP-61027)	1976	849,649
Fred Hutchinson Cancer Research Center (NO1-CP-61059)	1973	681,653
Hawaii, Research Corp. of the University of (NO1-CP-53511)	1971	483,000
Iowa, University of (NO1-CP-43200)	1973	1,437,563
Michigan Cancer Foundation (NO1-CP-61028)	1973	*
New Mexico, University of (NO1-CP-33344)	1973	792,976
Utah, University of (NO1-CP-43382)	1973	510,843
Yale University (NO1-CP-33235)	1973	359,914

*No funds added in FY 81, annualized expenditures are $1,783,168.

CONTRACT NARRATIVE

COMPUTER SCIENCE SECTION

GEOMET, Incorporated (NO1-CP-81019)

Title: Biomedical Computing, Design and Implementation

Contractor's Project Director: Mr. Richard Allen

Project Officer (NCI): Mr. James E. Larson

Objectives: This contract provides computer systems analysis and computer programming support to the Biometry Branch of the Field Studies and Statistics Program. The contractor is developing a library of statistical and data management computer programs for use in the collection and analysis of cancer incidence and end results data.

Major Findings: The contractor is providing continuing data processing support to Biometry Branch investigators. Computer systems and programs and related products are developed by the contractor in response to requests from these investigators. This year the contractor's level of effort averaged twenty-six person-years.

Significance to Biomedical Research and the Program of the Institute:

See Project Reports, NCI, Field Studies and Statistics Program, Biometry Branch.

Proposed Course: This contract terminated on June 30, 1980, and the effort recompeted with an award expected by July 1981 at an estimated annual level of $1,078,000. The need for these services is expected to continue indefinitely, although the level of support provided to each organizational area, as well as the total level of effort, are expected to fluctuate significantly over time with changing program requirements.

Date Contract Initiated: 9/28/78

Current Contract Level: $980,000

CONTRACT NARRATIVE

BIOMETRIC RESEARCH AND ANALYTIC STUDIES SECTION

HEALTH INSURANCE PLAN OF GREATER NEW YORK (NO1-CP-43278)

Title: Evaluation of Periodic Breast Cancer Screening with Mammography and Clinical Examination

Contractor's Project Director: Mrs. Wanda Venet

Project Officer (NCI): Dr. Philip C. Prorok

Objectives: A. To determine the frequency with which a screening technique using mammography and clinical examination can detect early breast cancer.

B. To establish whether such screening holds substantial promise for improving survival rates among women with newly diagnosed breast cancer and for lowering breast cancer mortality in the female population.

C. To investigate, through a prospective study of women screened, the relationships of a wide range of parameters to the development of breast cancer.

Methods Employed: This is a combination statistical-epidemiological-medical study in which a study population receiving clinical examination plus mammography at annual intervals and a control group of approximately 30,000 women each are being routinely followed for breast cancer experience through internal Health Insurance Plan files and through review of death certificates. Details of the techniques are given in a paper describing the methodology and initial findings of this study entitled "Evaluation of Periodic Breast Cancer Screening with Mammography" by Sam Shapiro, Philip Strax, and Louis Venet, JAMA 195: 731-738, 1966. The study is now in the long term follow-up phase.

Major Findings: Findings to date strongly suggest the usefulness of annual screening which includes clinical examination plus mammography. Over a 13-year period of follow-up, the study group of women has about one-third less mortality from breast cancer than those in the control group. This reduction in mortality appears to be concentrated among women over 50 years of age at entry into the study. The differential between control and study cases in fatality from breast cancer is almost entirely due to the exceptionally low rate among the cases detected through screening. Both the clinical examination and mammography contributed to this favorable situation, but the magnitude of the independent contribution of each modality is difficult to determine. In examining various risk factors for the women who appeared for an initial screening, the following women were found to have a 20-30% increase in risk of breast cancer: higher educated women, Jewish women, and unmarried women. Increasing age at first pregnancy, low gravidity, and menarche at ages under 15 are associated with a 50-100% increase in risk.

Significance to Biomedical Research and the Program of the Institute: Early diagnostic procedures offer considerable promise for the secondary prevention and potential cure of cancer. It appears that the combination of clinical examination and mammography is such a procedure and is being extended to other

populations. The role of mammography alone in this process is being investigated, as is the long-term impact of screening on breast cancer mortality.

Proposed Course: The screening part of the study is complete and the emphasis from now on will be on follow-up and data analysis. The women will be followed for as long a time period as is necessary to determine the long-term impact on mortality from breast cancer. All cases of breast cancer will be followed for at least 15 years. The current contract year is the first of an initial five year period for the long-term follow-up of all 60,000 women in the study. Such follow-up will allow quantification of the long term reduction in breast cancer mortality as a result of screening. During the current year, consideration will be given to including in this study an investigation of the risk of low dose ionizing radiation exposure to the breast in inducing breast cancer.

Date Current Contract Initiated: May 1, 1981

Current Annual Level: $400,000

CONTRACT NARRATIVE
CLINICAL AND DIAGNOSTIC TRIALS SECTION

INFORMATION MANAGEMENT SERVICES, INC. (NO1-CP-01025)

Title: Computer Services

Contractor's Project Directo: Janis Beach

Project Officer (NCI): Donald K. Corle

Objectives: This program provides system design and computer programming support for projects in the Clinical and Diagnostic Trials Section.

Major Findings: The contract was initiated on July 11, 1980 and replaces contract NO1-CP-71010 previously held by IMS. IMS periodically edits and updates data bases for studies of prostate, lung, brain, testis, and breast cancer. IMS personnel have written most of the computer programs used for managing these studies.

IMS personnel have worked closely with Drs. Gail and Rubenstein in preparing detailed reports concerning the five randomized clinical trials of the DCT Lung Cancer Study group. IMS personnel have attended the biannual meetings of this group to provide technical advice concerning forms design and data processing.

IMS personnel have continued to work with Drs. Green and Byar in developing computer programs for interactive statistical analysis. New programs for selecting subjects and matched controls were completed this year and improvements were made to existing programs.

IMS completed support for the DCCP Hospice Demonstration Project and Psychological Aspects of Mastectomy Study for which Dr. Muenz is the statistician. IMS generated the necessary computer analysis for the final publications on these studies.

Other projects for which IMS has given technical programming support include a study of geriatric blood specimens, a study of mycosis fungoides, a study of radiotherapy for prostate cancer involving three medical centers, and an EORTC bladder cancer study.

Proposed Course: IMS personnel have worked with the Section for all of Fiscal Year 1981. The contractor has been very responsive to the needs of the Section. The contractor's major responsibility will be in the development of editing, file maintenance and statistical programs for the research Projects conducted by the Section.

Date Contract Initiated: July 11, 1980

Current Contract Level: $360,000 FY 1981

CONTRACT NARRATIVE
DEMOGRAPHIC ANALYSIS SECTION

ISRAEL CENTER FOR REGISTRATION OF CANCER AND ALLIED DISEASES (NO1-CP-33351)

Title: Continued Cancer Registration and Selected In-Depth Analyses

Contractor's Project Director: Dr. Leah A. Katz

Project Officers: Dr. John L. Young, Jr.
Mr. William I. Lourie, Jr.

Objectives: With primary focus upon the effective functioning of the Israel Cancer Registry (ICR) in the collection of high-quality data of interest to NCI's Surveillance, Epidemiology and End Results (SEER) Program, (1) to continue the country-wide registration of cancer patients, (2) to utilize the data in a variety of statistical and epidemiological studies, (3) to acquire additional information from other pertinent record sources for use in formulating or testing hypotheses and in planning for collaborative studies, (4) to aid in implementation of field epidemiological studies including case-control studies which involve cooperation with medical specialists and other organizations, (5) to increase quality control of collected data and its classification by paralleling the methods of the SEER Program, (6) to submit original data to the SEER staff for NCI analysis in addition to local analyses, (7) to attempt recruitment of professional epidemiological personnel and to continue the technological upgrading of processing data in a confidential manner so that ICR's information may be used expeditiously by the health, medical and educational professionals of Israel, as well as epidemiologists throughout the world.

Major Findings: Using other national record sources, the ICR obtains information on country of origin, date of immigration, date and cause of death. Precise therapy details, identification of the cancer patient's demographic characteristics and the primary site, histology, and extent of disease of the tumor are obtained from medical records. An important recent advance has been a more effective liaison with the Population Register so that periodically an updated set of microfiche by name and by register number is received by ICR. This permits a more accurate determination of vital status and of the date of death and, as a result, a reduction in the number of patients "lost to follow-up" and, thus, the more timely production of survival data. In addition, the Population Register is the major source of confirmatory data concerning the origins of the various ethnic or cultural subgroups of the Jewish population as well as other immigrants. Differences in these subgroups in cancer incidence and/or mortality constitute the major epidemiological value of the ICR to NCI. In an "Epidemiological Review of Breast Cancer in Israel" Katz, Steinitz and Sela looked at ICR data for the 17-year period 1960-76 by five population subgroups and three time periods. They intensively analyzed the 12-year period 1961-72 by regions of birth for Jews born abroad and by three 4-year time periods. With approximately 1,000 new cases of female breast cancer per year, there was an increasing incidence from 1960 to 1976 in all age groups and in all populations, but with lower rates and lower median age in non-Jews. The peak incidence in all instances was observed after menopause. The greatest increase occurred in the previously very low incidence group of Jews born in Asia/Africa. The incidence remained almost unchanged for the Europe/America born group. The incidence rates ranged from a high among Ashkenazi Jews of Middle and Eastern Europe through the Sephardic Jews of Southern Europe and Turkey to a low among the North African and the other Asian Jews.

The greater availability of the Population Register's identity data facilitates an ongoing study on risk of cancer in families through a more precise identification of second generation immigrants as a separate group.

Steinitz, Katz and Ben Hur noted that in contrast with the Third National Cancer Study data for the USA, a higher male breast cancer incidence is demonstrated for Israel. The sex ratio is lower in Israel than in the USA although the number and mean age of male breast cancers are surprisingly similar.

Collaboration with the faculty of Haifa Medical School has permitted an expansion of the ongoing study of the gradient in lung cancer incidence which increases from Jerusalem to Tel-Aviv to Haifa. Occupational histories are being obtained by interview of lung cancer patients at the Thoracic Surgery and Oncology Departments of Rambam Hospital.

Geographical regions within the country are now routinely being coded and utilized to monitor cancer incidence by district, locality, primary site, sex and age. Data are utilized by the Health Ministry in planning for and providing services to the cancer patient.

Significance to Biomedical Research and the Program of the Institute: The Israel Tumor Registry has incidence data available from 1961 forward. In December 1980, a data tape containing information on 102,582 cases diagnosed between January 1, 1961 and December 31, 1978 was submitted to NCI. These cases are being converted to the SEER Program codes and format for analysis and comparison purposes. Of immediate interest are comparisons of incidence rates for various ethnic groups residing in Israel (including migrants from the United States) with rates for ethnic groups in the United States; a comparison of U.S. and Israel histologic patterns of cancer incidence within primary site groups; and a comparison of trends in cancer incidence in the two countries. While these analyses are being conducted by NCI, contract staff in Israel (in addition to maintaining the day-to-day operations of the registry) will devote themselves to several detailed analyses: cancer incidence in Israel by geographic area (district), cancer incidence in second generation immigrants, and the analysis of multiple primary tumors. It is now anticipated that these and other analyses will be published as an NCI publication (monograph).

Proposed Course: Funding is being planned through March 1984. This should allow sufficient time to complete the above analyses and to prepare a monograph. Currently, 65% of the costs of the Israel Tumor Registry are funded through NCI. During this funding period, the Registry will be encouraged to find other sources of funding.

Date Contract Initiated: June 1973

Current Contract Level: $137,500

CONTRACT NARRATIVE

OFFICE OF THE CHIEF

KUAKINI MEDICAL CENTER (NO1 CP 61060)

Title: Study of Cancer Among Japanese Migrants

Contractor's Project Director: Dr. Abraham Nomura

Project Officers (NCI): Dr. Earl S. Pollack, Dr. James Murray and Dr. David Levin

Objectives: The purpose of this contract is to collect data bearing on the reasons for the differences in cancer incidence between Japanese on the home islands and the Japanese migrant populations in Hawaii. The objective is to sort out those aspects of common cancers which may be genetically involved and those which may derive from aspects of the environment or some mixture of the two.

Methods Employed: Standard demographic techniques have been employed, including case-control and cohort studies. The matched case-control technique is particularly useful in this kind of study. Pathology protocols have been completed for cases in the study series for several sites (stomach, colon, rectum) and considerable emphasis has been placed on the correlation of the epidemiological and pathology findings. A cohort of Hawaiian Japanese males assembled by the National Heart, Lung and Blood Institute for studies of cardiovascular disease has been examined for information relevant to gastrointestinal cancers and their subsequent cancer morbidity experience is being monitored.

Major Findings: An analysis of the incidence of specific cancers in relation to serum cholesterol levels at the time of first examination was completed. It revealed that an inverse relationship between cancer incidence and serum cholesterol level existed only for colon cancer but not for any of the others. It manifested itself by a very high rate among those with serum cholesterol levels under 180 and lower rates for all of the other serum cholesterol groups. Similar findings were noted among the Japanese in Hiroshima and Nagasaki and among those being followed in the population of Framingham, Massachusetts.

The Biometry Branch staff has begun an anlysis of cancer incidence in relation to alcohol consumption as measured through the dietary interview at the time of first examination. The project is now in the process of including hematopoietic cancers in the surveillance because of their possible association with alcohol intake. In addition, the surveillance records are being modified so that stage of disease at time of diagnosis can also be included.

A number of special studies are being carried out. These include a recoding of the 24-hour diet recall questionnaire for colon cancer cases and two controls per case to assess the relationship between these cancers and vitamin A and vitamin C intake. Nitrite levels in saliva from gastroscopy subjects are being assessed to study the possible relationship between nitrite levels

and intestinal metaplasia of the stomach. The study of mutagens in human feces is continuing in a attempt to identify mutagens that might be related to the occurrence of colon cancer.

Significance to Biomedical Research and the Program of the Institute: If the studies in these migrant populations elucidate the environmental factors involved in the incidence of certain forms of cancer, and indiciate those that are predominately environmental and those which are predominantly genetics, then it may be posible to apply these findings to the United States population for purposes of cancer prevention and cancer control.

Proposed Course: The staff of the Biometry Branch will continue to carry out some analyses of the longitudinal data. However, discussions with the principal investigator have been initiated to explore the possibility of converting the project to a grant. Since the involvement of NCI staff in the research is not extensive, this may be a more appropriate course for the project.

Date Contract Initiated: June 1971

Current Annual Level: $542,193

LOUISIANA STATE UNIVERSITY MEDICAL CENTER (NO1-CP-53521)
FUNDACION PARA LA EDUCACION SUPERIOR (NOa-CP-33286)

Title: Studies of Epidemiology and Geographic Pathology of Cancer

Contractors' Project Directors: Dr. Pelayo Correa (NO1-CP-53521)
Dr. Carlos Cuello (NO1-CP-33286)

Project Officers (NCI): Dr. James Murray and Dr. David Levin

Objectives: To conduct pathological studies of premalignant lesions in New Orleans and Colombia, and to correlate findings with incidence and pathology data from other communities; to conduct laboratory experiments to elaborate and test hypotheses dervived from field studies.

Methods Employed: Cancer registries are being supported to provide the necessary baseline data for these studies. Standardized procedures have been developed for examining autopsy and surgical specimens for premalignant lesions. Some of the labortory work at LSU involves testing for mutagenicity of chemicals in the digestive tract.

Major Findings: The laboratory work in New Orleans includes a comparative study of the mutagenic activity in fecal specimens from polyp-bearers and controls. All the information will be collected by April 1981 and analyzed. Three years of cancer incidence data have been collected by the new registry in La Paz, Bolivia, and sources of continuous support are being sought for this uniquue registry. The findings from the first two years of data were presented at a recent symposium on cancer epidemiology. Incidence data at the cancer registry in Cali, Colombia are being analyzed for time trends for each cancer site. The Cali registry has been successful in finding sufficient local support for its continued operation after NCI contract support ends in April 1981. Multidisciplinary studies of the clinical, dietary, biochemical and

histopathological aspects of preneoplastic gastric dysplasias in high risk areas of Colombia are being completed this year. The results of an extensive nutrition survey are currently being analyzed and evaluated.

Significance to Biomedical Research and the Program of the Institute: This research on the etiology of stomach and colorectal cancer may lead to the development of useful preventive measures for these diseases.

Proposed Course: All contract research in New Orleans and Colombia will be completed in April 1981 as directed by NCI. Support for the registry in La Paz, Bolivia, will end in April 1982.

Dates Contracts Initiated: January 1, 1975 (NO1-CP-53521)
June 29, 1966 (NO1-CP-33286)

Current Annual Level: $10,500 (NO1-CP-53521)
$ 0 (NO1-CP-33286)

MINNESOTA, UNIVERSITY OF (NO1-CP-91021)
BERGEN, UNIVERSITY OF (NORWAY) (NO1-CP-91043)

Title: Analyses of Prospective Studies on Diet, Cancer and Other Diseases in Selected Migrant Populations (Norwegian)

Contractors' Project Directors: Dr. Leonard Schuman (NO1-CP-91021)
Dr. Erik Bjelke (NO1-CP-91043)

Project Officers (NCI): Mr. Joseph Scotto
Dr. John Gart

Objectives: To determine the reasons for difference in cancer incidence and mortality in sedente populations and migrant populations of the same ethnic and genetic composition. To pursue leads relative to diet variations among the two population groups.

Methods Employed: Prospective follow-up studies of two basic cohorts (one in the USA and one in Norway) are being conducted. Dietary and demographic data obtained from responses to questionnaires distributed in 1966-67 form the original baseline data to be compared with subsequent histories of disease (via cause of death information from death certificates in the USA, and cancer registration as well as information on deaths from all causes in Norway).

Major Findings: At this juncture, there are more reports and preliminary analyses from the Minnesota Lutheran Brotherhood study than from the Norwegian counterpart. There were 467 cancer deaths among 17,818 respondents during an 11 1/2 year follow-up of men insured by the Minnesota-based company. Cancers of the digestive system accounted for 167 deaths. Deaths from all causes totaled 2,305, with 1,025 due to heart diseases. Demographic comparison of the Lutheran Brotherhood study with the general population of United States males indicated that these respondents were (a) younger, (b) less likely to use alcoholic bevverages, (c) less likely to smoke cigarettes, (d) more likely to have a higher socio-economic standard, (e) more likely to live in rural farming areas, and (f) more likely to live longer than the

general population of U.S. males. A summary of findings, many of which are not conclusive at this time, include the following.

- There is an increased risk for colon cancer among persons with large consumption of fresh/frozen fish, smoked/salted ham or pork.
- The risk for colon cancer is enhanced for heavy users of meats/fats only when there is a corresponding low use of vegetables or grain fiber.
- Beer consumption appears to be positively associated with colon cancer mortality.
- There were no significant associations between alcohol consumption and mortality from cancers of the rectum, stomach, lung, bladder, prostate or leukemia.
- Positive associations were found between stomach cancer mortality and consumption of cooked cereals.
- Milk consumption was positively associated with stomach cancer mortality.
- There was no clear relationship between vitamin C index and stomach cancer mortality.
- Consumption of total meat, beef and fresh pork ham was not related to stomach cancer.
- There was a suggestive positive association between stomach cancer mortality and consumption of chicken, bacon/side pork, smoked/salted ham or pork, salted fish and total fish.
- There was a negative association between cancer of the pancreas and total meat consumption.
- Cancer of the pancreas mortality was positively associated with alcohol consumption, when controlling for the effects of age and cigarette smoking.
- Vitamins A and C may be protective against lung cancer, and perhaps stomach cancer and rectal cancer. The Minnesota study shows a possible increased risk between dietary vitamin A and colon cancer or pancreas cancer. These findings are highly tentative at the present time.

New data from the comparison prospective study conducted in Norway are now ready for preliminary analyses. There were 16,713 respondents to the Norwegian dietary questionnaire which are being analyzed. Among these, 1,577 new cases of cancer were diagnosed (1,357 among males) and 900 cancer deaths (793 among males) were recorded during 11 1/2 years of study follow-up. Of the gastrointestinal cancers, there may be enough cases to conduct meaningful analyses for stomach, colon, rectum, and pancreas with 135, 77, 52 and 50 cases, respectively, among males. Among other cancers of high frequency among males, those of the lung (170 cases) are also being studied concurrently. Several analyses look at specific dietary exposures, such as alcohol consumption and smoking habits and include all cancer sites in the analyses. The inclusion of incidence cases in the Norwegian data base make this study a more powerful one compared to the Minnesota study where only death statistics may be evaluated. The Norwegian study also includes 259 deaths among males from gastrointestinal cancers: stomach (118), colon (43), rectum (36), pancreas (48), and other digestive organs (16). As expected, there are many more stomach cancer deaths among Norwegians than among U.S. migrants from Norway. Deaths from all causes among the Norwegian cohort amounted to 4,107 of which 2,245 were from heart disease and other circulatory system diseases, including hypertension and cerebromacular diseases.

Preliminary indications from the Norwegian data tend to corroborate the Minnesota findings for vitamins A and C with respect to lung cancer and the positive association of beer consumption with colon cancer. Some of the Minnesota findings for pancreatic cancers and stomach cancers may, however, be at variance with the Norwegian data.

Proposed Course: During the past year computer files were generated in Norway to accommodate the analyses of the effects of diet on cancer incidence and mortality. We are now beginning to make use of specific statistical methodologies developed at NCI and programmed for computer analyses in Norway. With the limited amount of Norwegian staff trained in both statistics and epidemiology, it is anticipated that more direct NCI involvement will be needed. Collaborative papers including authors from the NCI, Norway, and Minnesota will be written on several topics, e.g., dietary factors and lung cancer, and alcohol consumption and cancer mortality. Because of the many cases of cancers from nondigestive sites, we project that future analyses will include more detailed studies on cancers of the prostate (263 cases), urinary bladder (95), skin (208), leukemia and lymphoma (105). Also, expanded analyses on specific factors cited in the recent literature, such as coffee drinking, will be pursued for certain cancers such as bladder and pancreas.

Future plans also include the possibility of re-interviewing, via mail questionnaire, the respondents of the Lutheran Brotherhood study who are still alive. This will provide information on significant changes in dietary habits during the past 10 to 15 years.

Significance to Biomedical Research and the Program of the Institute: This project is one of a few prospective, long-term epidemiologic studies designed to discern the influence of diet as well as other environmental factors on the incidence and morrtality of cancer. Isolation of these environmental elements will be useful in providing new leads on cancer etiology and in providing guidelines for preventive measures.

Dates Contracts Initiated: June 1966 (NO1-CP-91021)
September 1979 (NO1-CP-91043)

Current Annual Level: $129,132 (NO1-CP-91021)
$ 82,186 (NO1-CP-91043)

SOUTHERN CALIFORNIA, UNIVERSITY OF (NO1-CP-91027)

Title: Study of Cancer Incidence in the South Pacific

Contractor's Project Director: Dr. Brian E. Henderson

Project Officer (NCI): Dr. Earl S. Pollack

Objectives: To develop a comprehensive cancer incidence reporting system for the South Pacific, including Melanesia, Polynesia and Micronesia, so that incidence rates for the various ethnic groups in those islands can be compared with those of their counterparts in Hawaii and with incidence rates in general elsewhere in order to generate additional hypothesis regarding cancer etiology.

Methods Employed: Working through the South Pacific Commission as well as directly with personnel on the island, develop and improve the reporting of all cancers occurring among residents of the South Pacific islands. Data are collected on a standard form and sent to the University of Southern California (USC) for processing. Tabulations and analyses will be carried out by USC staff and data tapes will be sent to NCI for further analyses.

Major Findings: Data collection is now being carried out with an added impetus resulting from a meeting of a consultant to the project with the Program Directors in all of the countries that are members of the South Pacific Commission. Cancer incidence data have been tabulated by the project staff for the various geographic areas of Papua, New Guinea and comparisons have been made through proportionate incidence rates. It is not possible at this time to determine the degree of completeness and accuracy of these data. Only fragmentary data have been obtained thus far from other areas.

Significance to Biomdical Research and the Program of the Institute: The project is highly relevant to work now going on in the Biometry Branch for at least two reasons: (1) it will provide cancer incidence data on a set of unique population groups in a way that will permit comparison with those for populations now being studied through the SEER Program; (2) these particular population groups represent countries of origin for many of the population groups in Hawaii and therefore provide an opportunity for futher formulation of specific hypotheses, although the identification of these groups as migrants to Hawaii is indeed difficult.

Proposed Course: It is anticipated that further tabulations will be produced this year and that at the termination of the contract in September 1981 a set of data tapes on available registry data in the south Pacific areas will be provided to NCI for further analysis.

Date Contract Initiated: September 14, 1979

Current Annual Level: $50,382

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ANNUAL REPORT
CLINICAL EPIDEMIOLOGY BRANCH
OCTOBER 1, 1980 THROUGH SEPTEMBER 30, 1981

Clinical epidemiology is a form of observational research in which one must make the most of natural occurrences to determine the causes and mechanisms of disease. Our approach is not traditional, but has proved to be continuously productive. Specifically, the Clinical Epidemiology Branch (CEB) seeks peculiarities in the occurrence of cancer in persons, families, communities or industries that may lead, in conjunction with recently developed laboratory research, to new knowledge of biology. In this way, study of human disorders may illuminate areas for which no animal models are yet known. Such observations may lead to new concepts of early detection and prevention.

Staff:

Ms. Nancy Strickman joined the Branch in mid-December 1980 to provide much needed epidemiologic talent in our neurofibromatosis studies. Her temporary duty as a Commissioned Officer came to an abrupt end on April 13, 1981, when her assignment was not automatically renewed by the PHS, as expected. The professional staff now consists of 6 physicians trained in pediatrics, internal medicine, oncology, hematology, human genetics and/or epidemiology; a geneticist, biostatistician, a masters-level epidemiologist and a computer specialist.

Inter-Institute Human Genetics Clinic:

Dr. D.M. Parry of our Branch has played a leading role in establishing the Inter-Institute Human Genetics Clinic. During its second year, about 300 persons representing 60 diagnostic categories were seen, 32 percent of them by members of our Branch. The Clinic provides a setting for exchanging information and prompt consultations with the staff of other participating Institutes of NIH. The patients are the subjects of case-presentations, and visiting lecturers bring knowledge of recent developments from their research, appropriate to the Clinic's function and the teaching of the staff and medical students on elective in human genetics at NIH. The Clinic serves as a center for obtaining specimens for laboratory studies concerning diagnosis and for studying the biology of cancer and other diseases. Among the patients seen there during the year in connection with oncology, emphasis was on neurofibromatosis. The array of patients with this disease seen during the past year has given rise to a variety of studies of this previously unfashionable subject for research.

Neurofibromatosis (NF):

Our interest in NF, smoldering over the years, received great impetus from the first conference on the subject ever held (December 1979, organized by Dr. J.J. Mulvihill and V.M. Riccardi, sponsored by our Branch and the newly established National Neurofibromatosis Foundation). The proceedings are soon to be published (74).

At about the same time the Branch established that two forms of cancer, non-lymphocytic leukemia and rhabdomyosarcoma, occurred excessively with NF. These associations are puzzling because the cells involved are now known to be derived from the neural crest, as the other elements of the syndrome apparently are.

In conjunction with the National NF Foundation, Dr. J.L. Bader and Ms. N. Strickman have conducted a study of self-administered questionnaires completed by 156 NF-affected members of the Foundation to evaluate the disease in a sample not drawn from a hospital. The family histories identified a total of 318 persons with NF. The results to date indicate that neural cancers are more frequent than usual, the risk being greater in females, but other cancers in broad categories were not demonstrably more frequent than in the general population (78). A life-table for persons with NF is being developed, along with a study of the causes of death. An international study of childhood cancers registered for other purposes showed that at least one percent were known to have NF. In the Inter-Institute Genetics Clinic 65 persons were examined for NF in 1980, including a pair of identical twins only one of whom has NF which she transmitted to 2 of her 3 children (80). The parents of the twins did not have NF. The occurrence of clinical NF in only one of the members of a genetically identical pair points up the need for a biomarker to detect subclinical NF, as in skipped generations of families with the disease, children of NF patients, and persons or families with the neoplastic complications but not with classical signs of the disease. Among other topics that have emerged for study are segmental NF (localized area of body affected), drugs or physiologic changes that accelerate or decelerate the disease and genetic linkage studies through laboratory determinations to seek the locus (loci) for the NF gene.

Bedside Etiology of Cancer:

The Branch has long been a proponent of clinical observations that provide clues to the etiology or biology of cancer. One of its greatest successes during the past two years is the identification of "trilateral" retinoblastoma--in which both eyes and the pineal ("the third eye") are affected, presumably because all 3 contain retinocytes. The entity was registered in the literature through a letter to Lancet (3), and a comprehensive description of the disorder has now been prepared for publication. It concerns 11 patients, most of them diagnosed recently in the U.S. Subsequently, we learned of 3 additional cases upon diagnosis. Some of the children have unusually located second primary tumors which may possibly involve retinal anlage cells wherever they may be (i.e., a form of multicentric cancer rather than pleiotropism). Dr. Bader, who has led these studies, suspects that cases are increasingly being identified because CAT scans are showing pineal tumors previously mistaken on clinical grounds for metastases to the brain. This suggestion may herald a new era in cancer etiology, detection and treatment, as indicated by recent reports of very early diagnosis of neuroblastoma and of Wilms' tumor in high-risk patients whose cancers were not detectable by older diagnostic procedures.

Accessory Nipples and Renal Cell Carcinomas:

During a fellowship in Oncology at Georgetown University a year ago, Dr. E.A. McKeen of our Branch made an exceptionally alert bedside observation when she noted that two persons with renal cell carcinoma had accessory nipples. She and Dr. J.J. Goedert of the EEB, examined 32 other Washington-area patients with the tumor and found that 6 had accessory nipples as compared with only 1 in a series of matched controls (27). The embryology of accessory nipples is poorly defined, so their association with renal cancer is not readily explained. Other studies are in progress to clarify the link between the two disorders.

Leukemia with Congenital Malformations:

Surprisingly, the number of malformation syndromes that apparently predispose to leukemia continues to increase. Dr. P.A. Gilman has been tracking these in the literature and as unreported cases. She has found 3 unreported cases of leukemia plus 4 reported with Shwachman's syndrome (exocrine pancreatic deficiency with blood dyscrasia); 4 leukemics and 2 children with hepatocellular carcinoma and Diamond-Blackfan syndrome (congenital hypoplastic anemia, some of whom have the Turner phenotype); 2 leukemics reported and 1 unreported with Kostmann's syndrome (genetic infantile granulocytopenia), and 1 other unreported case with non-Hodgkin's lymphoma. Also, leukemia has been reported in probable excess with Rubinstein-Taybi (broad thumb-hallux syndrome), Poland's syndrome (absence of the pectoralis major muscle with syndactyly), neurofibromatosis, and perhaps in Marfan's syndrome. These concurrences may have more than one explanation, but it may be etiologically worthwhile to consider what they have in common. Several are being studied cytogenetically.

Studies in Boston:

Dr. F.P. Li continues to make exceptional etiologic observations at the Sidney Farber Cancer Center in Boston. During the year, a child thought to be at increased risk of leukemia developed the neoplasm as predicted on the basis of family aggregation of cancer, ataxia and absence of chromosome 7. A manuscript describing this new development in the family has been submitted for publication.

In another family, autosomal recessive inheritance was a possible explanation of acute myelogenous leukemia in brothers both of whom had multiple polyposis of the colon with adenocarcinoma (28). Another patient with polyposis of the colon and brain tumor (Turcot's syndrome) developed acute leukemia after cranial radiotherapy, possibly related to in vitro sensitivity of her fibroblasts to ionizing radiation. The same sort of sensitivity was found in 2 sisters who developed breast cancer after radiotherapy for Hodgkin's disease (42). Presumed radiogenic breast cancer was also observed in 4 children given radiotherapy for Wilms' tumor or bone sarcoma (42). These cases were found in a survey of 910 survivors of childhood cancer. Colon cancer of early onset has been observed in a third survivor of Wilms' tumor given radiotherapy (37). Radiation as a cause of colon cancer has not previously been as convincingly demonstrated.

Other findings of long-term survivors of childhood cancer include 1) high ovarian failure following prepubertal radiation to both ovaries; 2) a high frequency of thyroid dysfunction with elevated TSH after high-dose radiotherapy; 3) a high frequency of late effects, especially kyphoscoliosis after radiotherapy in the first month of life; and 4) an excess of small-for-date babies born to women given radiotherapy for Wilms' tumor.

Of great interest is a follow-up study by Drs. Li and Fraumeni of families they first described in 1969 in which 2 children had soft-tissue cancers and other relatives had a variety of cancers, especially female breast cancer early in life. In the 12 years that have elapsed, 16 cancers have developed (9 of them of the breast) as compared with 0.5 cases expected.

BEST DOCUMENT AVAILABLE

Reproductive Performance Among Survivors of Childhood Cancer:

In collaboration with members of the Biometry Branch, Dr. J.J. Mulvihill and Ms. Margot R. Hanson of our Branch are conducting a study of the reproductive histories of persons who had cancer in childhood 5 or more years ago and who are at least 18 years old now. Three of the 5 centers under contract with the Biometry Branch are in the SEER program (California, Connecticut and Iowa), the other two being the University of Kansas and the University of Texas System Cancer Center in Houston. Each has 270-903 (Total=2854) candidates for study and comparison with sibling-controls. The objective is to determine the effects if any on reproduction, of the cancer or its treatment. The data collection by interview will end after 2 years, in the Fall of 1982.

In related studies, 28 children of 448 patients treated for cancer at NCI have been examined and no impairment of reproduction has been found. Also, a collaborative study is being made of the 137 pregnancies of 66 women previously treated for Hodgkin's disease by physicians in Leukemia Group B.

Other studies:

Among more structured conventional epidemiologic research conducted by the Branch are chart reviews of Ewing's tumor for associated malformations, of about 120 patients with Diamond-Blackfan syndrome to define characteristics other than hematologic, and of children with sacrococcygeal teratomas for associated anomalies and family histories (Dr. Gilman). To evaluate workhistories from the Social Security Administration as compared with those obtained from the next of kin, study is being made of 150 men who died of mesothelioma, a cancer often due to asbestos exposure (Drs. Beebe, Spirtas and O'Conor). A prospective study is being initiated of soldiers who developed postvaccinal hepatitis in 1942 to determine if they suffered an excess mortality from hepatocellular carcinoma, with laboratory studies of sera from a sample of survivors to determine if infection was with hepatitis B (Dr. Beebe, et al). A multihospital chart-review of patients with chemodectomas has been completed and a report submitted for publication (Dr. Parry, et al).

A medical student, on elective with us, was first author of a report of a woman who had no liver abnormalities 14 years after repeated attempts at suicide using massive doses of aflatoxin (77). A medical resident, on elective with us, further developed two remarkable pedigrees with aggregation of lung/larynx cancer, with limb and dental anomalies in one kindred. In each family, a young adult has recently been found to have neoplasia after childhood radiotherapy to the anatomic region. Thyroid cancer occurred in one and squamous cell carcinoma of the face in the other.

Nine papers were published on original observations not related to cancer (1, 24, 25, 32, 34, 35, 44, 69, 70).

Radiation Studies:

Dr. G.W. Beebe, who is with the Branch as an Expert, has been an international leader in judging the effects of ionizing radiation in man. During the past year, he has published on epidemiologic evidence from the 2 main human studies with regard to mechanisms of carcinogenesis (10), given the Wade Hampton Frost Lecture at the annual meeting of the American Public Health Association on

what we know and what we do not know about radiation effects (16), lectured before the American Chemical Society on what we most need to investigate now and in the future, addressed the Council of the National Commission for Radiological Protection (15) on estimating somatic risks and factors that influence the size of the risk, and presented statistical considerations at a meeting organized by the American Statistical Association at the request of EPA.

Dr. Beebe was a primary contributor to the NAS report on the Biological Effects of Ionizing Radiation (BEIR III), completed in 1980 and published in 1981. He continues to participate in Dr. Fredrickson's "kitchen cabinet" on ionizing radiation, in NCI's group (BELLRAD) that meets occasionally to discuss present and future studies and possible organizational innovations concerning low-dose radiation research at NCI. He is also a member of the advisory groups for the fallout-exposed Marshall Islanders, and the Johns Hopkins study of nuclear shipyard workers. He attends a variety of other committee meetings concerned with radiation effects.

Chalk River Contract:

The contract with Dr. Malcolm C. Paterson's Group (Atomic Energy of Canada, Ltd.) is in its third and final year as a sole source. The purpose of the contract is to seek evidence of in vitro sensitivity of cells in culture from persons or families suspected of being unusually sensitive to X-rays, UV radiation and/or drugs, or with a marked familial or personal aggregation of cancer. During the year, about 30 specimens were studied. Among the findings was extreme sensitivity to MNNG in cells from female relatives of a woman with Gardner's syndrome (89), and a less marked sensitivity of cells from a child with bilateral retinoblastoma and pineoblastoma. Six cell lines from patients with melanoma were evaluated, and all six were sensitive to 4NQO and UV light, but not to gamma radiation (90). Skin fibroblasts from a family with remarkable aggregation of cancer (neurolemmoma, astrocytoma, osteosarcoma, medulloblastoma and acute lymphocytic leukemia, among others) revealed unusual radioresistance on culture (5). Another family with marked predisposition to acute myelogenous leukemia had somewhat increased radiosensitivity of skin fibroblasts in culture (6). The contract will compete for renewal when its sole source funding runs out.

Three-Dimensional Graphs of U.S. Cancer Mortality:

The potential value of 3-dimensional graphs to portray the U.S. cancer mortality by age and calendar year, 1950-1977, was described in 2 publications (29, 30), one of which illustrates how the baby-boom of the 1950's will increase by 75 percent the number of breast cancer deaths in 20 years among women 40-49 years old (30) if means for prevention or better treatment are not found. Use of the graphs for melanoma revealed a substantial increase in mortality rates over time. A marked increase has occurred in the number of deaths from testicular cancer among teen-agers in the baby-boom, who have reached the first age-peak in the natural occurrence of the tumor (85). A volume based on the almost 8 million cancer deaths in the U.S., 1950-1977, is almost ready for publication (47).

Other Computer Activities:

For an evaluation of the accuracy of death-certificate diagnoses of cancer, mortality data for 1971 from certain states and counties were provided to Dr. C. Percy of the Biometry Branch (Percy, C. et al: Am. J. Public Health 71:242-250, 1981). In response to a request from the Nuclear Regulatory Commission (NRC) concerning evaluation of the effects of nuclear fallout, data were provided for selected counties in Arizona, California, Utah and Nevada. In response to a request from the Virginia State Health Department concerning evaluation of the health effects of kepone, data were provided on biliary and liver cancer mortality by county in the state. In response to a request from EPA, annual cancer mortality data for New Jersey, 1960-1965, were provided to allow interpolation of data by color, omitted from the death-certificates in 1962 and 1963.

Computer services were provided to bring up to date a bovine leukosis/human lymphoma comparison originally published by Dr. Priester, a member of the Branch, in 1970. Three-dimensional graphs were made for childhood leukemia by single year of age, 1950-1977, and for all childhood cancer in that interval; the NRC was provided with software to map boundaries of the 3056 counties in the U.S.; other information concerning mapping systems have been given to several universities; a new black-and-white shading method for plotting the counties is under development; and tabulations have been made of toxic chemical spills in transit, using data obtained from the Department of Transportation.

Development of Epidemiologic Resources:

In addition to innovations concerning national cancer mortality data, the Branch has campaigned vigorously since 1968 for establishment of a National Death Index (NDI) which would greatly simplify follow-up studies of persons with various environmental exposures or diseases. In 1979 agreement was finally reached to establish NDI within the National Center for Health Statistics. Dr. Beebe has been on the advisory group for the data collection and usage. This includes the selection of techniques for matching the NDI data against study rosters. He has also been on the Working Group for developing a Continuous Work History Sample, based on data from the Social Security Administration (12, 13). The information sought would serve to evaluate mortality in relation to work history.

Veterinary Medical Data Program:

From 1961-1978, the Branch had a section on veterinary cancer, which devised a system for collecting data on all domestic animals discharged from 15 university veterinary hospitals in the U.S. and Canada. Among 1.6 million cases accessioned, 41,569 had tumors. A monograph with the tabulations was published during the past year (73), long delayed by the contractor for printing the volume. This publication terminates the Branch's continuous activities in studies of cancer in domestic animals.

Foreign Affairs:

Japan: A workshop on Differences in Lymphocytic Diseases between the U.S. and Japan was held in Honolulu, March 11-12, 1981. The meeting was organized by Drs. R.W. Miller and H. Sugano, with the assistance of Dr. P.A. Gilman of our Branch. A reciprocal relationship between lymphomas and autoimmune diseases, both of them lymphocyte-mediated, had been observed in U.S. males vs. females, and some evidence was known for a similar ethnic difference (e.g., U.S. vs. Japan). Rates for lymphoma are high in U.S. males and in whites, but low in U.S. females and Japanese. The opposite is true for systemic lupus erythematosus (SLE) and other autoimmune diseases. Lymphoproliferative disorders known to be rare in Japan are chronic lymphocytic leukemia and Hodgkin's disease under 30 years of age (i.e., the early age-peak is missing there). At the workshop the low rate for non-Hodgkin's lymphoma was revealed to be due to a near-absence of the nodular form of the neoplasm in Japan.

Lymphocytic diseases that occur excessively there are adult T-cell leukemia in Kyushu, mucocutaneous lymph node syndrome, Takayasu's aortitis, Hashimoto's thyroiditis, SLE, subacute necrotizing adenitis in Hokkaido, plasma cell dyscrasia with polyneuropathy and endocrine disorders (Takatsuki's disease), benign T-cell lymphodenopathy with peripheral eosinophilia and elevated IgE levels (Kimura's disease), and stomach cancer among persons with dermatomyositis.

A variety of new collaborations were suggested within and between the two countries and among the various specialists who participated. The workshop was sponsored by the U.S.-Japan Cooperative Cancer Research Program. Highlights of the meeting have been submitted for publication.

Radiation Effects Research Foundation: Dr. Miller served as a member of the Scientific Council of RERF at its annual meeting in Hiroshima, March 19-21, 1981, and participated in a Cancer Detection Workshop there for two days before the Council meeting. The most interesting new developments concerned the possible use of sonograms for routine screening of the upper abdomen (which have revealed a wide array of findings elsewhere in Japan), and a follow-up study at Hiroshima University of former mustard-gas workers (during World War II), among whom the number of respiratory cancers diagnosed has climbed from 49 in 1969 to 73 in 1981. The 49 cases were reported in 1969 under a contract from our Branch to put the study on a firmer epidemiologic basis.

Third International Conference on Environmental Mutagenesis: Two Branch members are planning to participate in the Conference in Tokyo and Mishima, September 21-25, 1981. Dr. Miller will speak on the role of the clinician in monitoring for diseases from chemicals in the environment, and Dr. Mulvihill will speak on epidemiologic aspects of ecogenetics in human mutagenesis.

NIH-Japan Panel on Environmental Mutagenesis: Dr. Mulvihill is serving as a member of this binational panel, which met in September 1980 in Karuizawa, Japan. Dr. Mulvihill's role is to increase the medical orientation of the panel.

People's Republic of China: From November 16-22, 1980, Dr. Miller participated as Dr. DeVita's alternate in the second annual meeting of the U.S.-P.R.C., Joint Health Committee in Tienjin to represent NCI interests in binational exchanges in oncology. The first exchanges under the federal program were to be between staff members of NCI and the Cancer Institute of the Chinese Academy of Medical Sciences in Beijing. High level administrative complexities on both sides prevented the program from moving forward during the year, to the dismay of our Chinese counterparts. The personal interactions so carefully nurtured outside the federal program since 1977 were disrupted earlier in 1980. Discussions during the Joint Committee meeting reestablished the spirit of mutual cooperation. Actually, the program in oncology has moved forward quickly under the program sponsored by the National Academy of Sciences in the U.S. (U.S. Cancer Delegation, with three members from NCI to the PRC for 22 days in 1977; a senior scholarship for Dr. F.P. Li of our Branch and his wife for 3 months in epidemiology at the Cancer Institute in Beijing in 1979; Visiting Lectureship in epidemiology for Drs. Li Bing and Zhang Yu-hui in the U.S. for 2 months [half of the time at NCI in 1980]; and a research project award to Dr. C-c. Ting of NCI and his wife to spend three months in immunology and map-making by computer in Beijing in 1981). In addition, Dr. J-y. Li of the Cancer Institute in Beijing is spending one year in the two NCI Epidemiology Branches (including 3 months with Dr. F.P. Li) under the auspices of IARC, and Dr. J-t. Tu of the Shanghai Cancer Institute is spending one year in the Biometry Branch, NCI, as a Visiting Fellow. Other Chinese at NCI during the year have not been involved with epidemiology or biometry.

At the Joint Committee meeting agreement was reached that 4 Chinese (2 in epidemiology) would each spend a year at NCI beginning in 1981 and 4 NCI staff members (one in etiology) would teach, demonstrate new techniques and/or engage in collaborative research for one month each in Beijing or Linxian where an epidemic of esophageal cancer is under study.

Dr. Miller has played a main role in planning these exchanges and published an article during the year, Cancer Epidemiology in China: Two Years of Progress (50). Since the last annual report, Dr. F.P. Li of our Branch has published on screening for esophageal cancer in 62,000 Chinese, cancer mortality maps in China (45), the incidence of childhood leukemia in Shanghai (43), the rarity of Ewing's sarcoma in China (46), acupuncture and possible hepatitis B infection (44), and projections for cancer mortality in China until the year 2000 (39).

Seveso, Italy: At the annual meeting of the International Advisory Committee on Seveso, concerning exposure to dioxin which escaped from a factory in July 1976, Dr. Miller served as a member and helped write the report evaluating the follow-up studies to date: no detectable excess of cancer or birth defects were detected. Chloracne was the only definite finding to date (Recommendations of the 4th Meeting of the International Scientific Advisory Committee for Seveso, January 10-14, 1981).

International Agency for Research on Cancer (IARC):

Drs. Miller and Mulvihill participated in an international conference convened in Cape Sounion, Greece, June 6-12, 1981, on host factors in cancer. Dr. Mulvihill spoke on clinical genetics of human cancer and Dr. Miller chaired a session on genetic and familial factors and helped prepare a summary of this portion of the meeting.

International Commission for Protection against Environmental Mutagens and Carcinogens (ICPEMC):

Dr. Mulvihill is a member of the Epidemiology Committee of ICPEMC, an organization that deliberates on matters of international importance concerning mutagenesis, carcinogenesis and their relation to one another. He participated in the annual meeting held on May 4-8, 1981, in Evien, France.

Cancer Communications:

The Childhood Cancer Etiology Newsletter, issued monthly since December 1973, continues to generate interest and research in the subject. It helps keep our staff and about 900 other people worldwide up to date on recent developments.

Lectures are frequently given by Branch members to audiences of various sorts (e.g., scientific leaders of NIH, scientific staffs of Branches in NCI or in other Institutes, at universities and hospitals, to other members of the health professions and occasionally to the public). A substantial number of analytical reviews and textbook chapters were published or accepted for publication during the year (2, 7-9, 26, 31, 36, 38, 40, 41, 49-68).

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04325-18 CEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Planning and Development in Cancer Epidemiology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI :	R.W. Miller	Chief	CEB NCI
OTHER:	F.P. Li	Head, Clinical Studies Section	CEB NCI
	J.J. Mulvihill	Head, Clinical Genetics Section	CEB NCI
	G.W. Beebe	Expert, Biostatistics	

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Epidemiology Branch, Field Studies and Statistics Program

SECTION
All units

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0.0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives of this undertaking are to originate new epidemiologic approaches to the study of cancer causation in man, to develop sources of data related to specific epidemiologic problems, and to stimulate epidemiologic research in other health agencies.

Opportunities for research in cancer epidemiology are based on questions arising from clinical or laboratory observations, on unusual groupings of cancer cases in the population, and on study of the characteristics of groups of persons prior to the onset of a specific type of cancer as compared with similar persons who have not developed the disease.

PHS-6040
(Rev. 2-81)

Project Description

Objectives:

1. To originate new epidemiologic approaches to the study of cancer causation in man.

2. To develop sources of data related to specific epidemiologic problems.

3. To stimulate epidemiologic research on cancer and other diseases.

Methods Employed:

The Program is based on:

1. Leads from animal experimentation, laboratory research and clinical observations.

2. Prospective studies (in retrospect) which relate cancer occurrence to events recorded prior to the onset of cancer in medical examinations obtained in a standard fashion from large numbers of persons (examples--clinical health-surveys, and military medical examinations).

3. Retrospective studies based on questionnaires obtained by personal interview or by mail, for a comparison of persons.

4. "Laterospective" studies which concern the detection from clinic records of the excessive concurrence of cancer with pre-existent disease, such as congenital defects or autoimmune disorders.

As specific epidemiologic questions arise from laboratory or clinical observations, sources of field data are developed to answer them. Conversely, the Branch seeks by its surveys to raise questions which can be answered by laboratory or clinical studies.

Major Findings:

Until a few years ago when the Privacy Act and human ethics committees were established, epidemiologists could abstract vital records and hospital charts at will. In our experience, over fifteen years, this approach produced a steady flow of important new information about the etiology and natural history of cancer. Never did we have a problem with privacy or ethics, but the new restrictions stopped our research along these lines. Instead, we have turned to clinical observations of peculiarities in cancer occurrence and explored their biologic nature through laboratory research performed under contract or collaboratively.

Significance to Biomedical Research and the Program of the Institute:

If the example set by the Branch is a good one, interest will be stimulated among medical scientists in the use of epidemiologic methods for their research. In consequence, there may develop a further recognition of the usefulness of office and hospital records for survey studies. With this would go an

appreciation for the need to adapt the standard medical records for epidemiologic research. These developments, in turn, could promote an interest in looking beyond the walls of hospitals and medical centers for opportunities in medical research.

Proposed Course:

We plan to continue and extend etiological studies of patients with cancer and of cancer families at NIH or elsewhere in the city or nation, and to encourage similar approaches internationally. Specimens from cases of interest will be obtained for laboratory studies using new procedures in an attempt to develop further understanding of factors that increase host-susceptibility to environmental carcinogens. New ways of portraying cancer mortality data will be undertaken to seek peculiarities in distribution which have etiological implications. Tests of hypotheses will be made as they arise from experimental or clinical observations.

Publications:

See Bibliography numbers: 2, 4, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 34, 39, 50, 51, 53, 54, 55, 56, 57, 58, 60, 61, 62

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04377-10 CEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Familial, Congenital, and Genetic Factors in Malignancy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI :	J.J. Mulvihill	Chief, Clinical Genetics Section	CEB	NCI
OTHER:	E.A. McKeen	Clinical Investigator	CEB	NCI
	D.M. Parry	Visiting Scientist	CEB	NCI
	R. Willis	Volunteer Medical Student	CEB	NCI
	M. Reardon	Research Assistant	CEB	NCI
	T. Goffman	Volunteer Physician	CEB	NCI

COOPERATING UNITS (if any)
Environmental Epidemiology Branch and various other clinical and experimental groups within NCI (especially Pediatric Oncology Branch), Atomic Energy of Canada

LAB/BRANCH
Clinical Epidemiology Branch, Field Studies and Statistics Program

SECTION
Clinical Genetics Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.6	0.9

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Study of genetic diseases with neoplastic manifestations and detailed investigation of families with high risk of cancer may help detect environmental and genetic influences in carcinogenesis, especially when appropriate laboratory assays are used.

Clinical recognition of accessory nipples in two patients with kidney cancer led to a case survey that found a 50-fold risk of accessory nipples in renal cell carcinoma patients. In vitro radiation sensitivity was unusually high in a family with acute myelogenous leukemia and a radiogenic rectal carcinoma, and unusually low in one with diverse malignancies. A family with Waldenstrom's macroglobulinemia had a broad clinical and laboratory spectrum of immune defects. Genetic consultations led to devising a generalized statistical method for designing family studies and to recognition of a new X-linked immunodeficiency syndrome with growth hormone deficiency. Guest lectures, literature reviews, and committee activities were done to stimulate similar research worldwide.

PHS-6040
(Rev. 2-81)

Project Description

Objectives:

To identify genetic factors and disorders associated with cancer, and promote similar studies worldwide. To document the occurrence of patterns of familial aggregation of neoplasms; to study selected families by genetic and laboratory investigations in an effort to elucidate carcinogenetic mechanisms and the degree to which heredity and the common familial environment contribute to the etiology of neoplasms. To distribute biologic specimens from selected subjects to laboratory investigators for etiologic studies by biochemical, cytogenetic, immunologic, viral, and tissue culture methods. To study similarly, patients with birth defects and other hereditable disorders that may predispose to malignancy.

Methods Employed:

Interview of patients with cancer or other diseases with respect to familial occurrences especially of cancer and birth defects, as well as prior medical and environmental history; documentation of history by obtaining appropriate vital records and hospital charts; collection and distribution of biological specimens from such families; review of hospital records of series of patients with selected congenital and genetic diseases or neoplasms. Invited lectures, reviews, and committee memberships provide for a stimulating research in cancer genetics.

Major Findings:

Reports published or in press in the last 12 months by the three permanent participants of this project comprise nine reports of original research [concerning laboratory findings in cancer families (3), case reports (3), clinical studies (2), and statistical methodology (1)] and eleven reviews, as well as eight abstracts for national meetings. Research reports involved 26 co-authors from the Environmental Epidemiology, Immunology, Pediatric Oncology, Medicine and Liver Disease Branches of NIH, Atomic Energy of Canada, and Georgetown University, and the Universities of California (Davis), and Cincinnati, (Seattle), as well as Montgomery and Mt. Holyoke Colleges, and Emory University.

Astute clinical recognition of accessory nipples in two patients with renal cell carcinoma was confirmed in a clinical survey that found accessory nipples in 19% of 32 renal cell carcinoma patients in contrast to 0% in 32 head and neck cancer patients and 0.5% in published population surveys. The relative risk was 5.0 together with last year's findings of a chromosomal translocation in a family with renal cell carcinoma, the prenatal origins of that adult tumor are, to our continued surprise, becoming conspicuous. Both findings could obviously be useful in a screening and surveillance effort, as well as in initiating studies to understand etiology.

Sophisticated laboratory methods were successfully applied in probing for carcinogenic mechanisms in three families. In two, clinical reports had already been made and the availability, by contract, of expert collaboration in radio-

biology gave new insights. In a family with diverse tumor types (sarcoma, brain cancer, breast cancer, and others), fibroblasts were unusually resistant to killing by gamma-radiation. The most radioresistant was a man with polycythemia vera and occupation exposure to radionuclides. In a sibship with acute myelogenous leukemia, fibroblasts of affected sibs were unusually radiosensitive in the range of heterozygotes for the ataxia-telangectasia gene, whereas the normal father and twin brothers showed the normal radiation response. Also unusually sensitive were cells from the mother, who died from rectal carcinoma, possibly attributable to radiotherapy she received for a cervical cancer 15 years earlier. A third interdisciplinary report described a family with Waldenstrom's macroglobulinemia, where two early cases were diagnosed on the occasion of our investigation, the occurrence of elevated IgM, thyroid disease, other autoimmune disorders, and similar HLA and B-cell alloantigens may reflect a hereditable abnormality in the immunoregulation of B-lymphocytes. In addition, preliminary findings were published on the immune status of various cancer families and the unusual in vitro sensitivity to chemicals known to interfere with nucleic acid metabolism in a family with the Gardner syndrome of colonic polyposis, jaw osteomas, and subcutaneous cysts, and the new hereditable dysplastic nevus syndrome.

After clinical researchers long involved in the study of hypertrophic cardiomyopathy sought our advice for a study of the genetics of the disorder, a generalized design strategy was devised for maximizing efficiency of family studies by eliminating marginally informative families, when a large number of families are available. Similar genetic consultations led to recognition of a new X-linked hypogammoglobulinemic immunodeficiency associated with growth hormone deficiency. The clinical recognition of this entity raised questions about the interactions between neuroendocrine and immune functions.

Other case reports pointed out a new syndrome of premature aging and pigmented nevi and the association of Hirschsprung aganglionosis with Aarskog syndrome. A final case report documents the lack of tumor 14 years after a laboratory worker attempted suicide by ingesting partially purified aflotoxin.

A book was co-edited on Neurofibromatosis, (von Recklinghausen's Disease), the genetic disorder of the Elephant Man, that seems understudied in view of its prevalance and the existance of advanced neurobiological and in vitro techniques that might be helpful if applied to the disease. In addition to contributing to a chapter on diagnostic criteria in neurofibromatosis, a definitive review of malignancy complicating neurofibromatosis was prepared for the volume. Other specific literature reviews addressed the potential carcinogenicity of the intrauterine device and the current status of the fetal alcohol syndrome. Five general reviews considered the epidemiology and etiology of childhood cancer, the possibility of cancer control through applying clinical genetics, and the clinical genetics of human cancer.

The stimulating research on the interaction of host and environmental determinants of human cancer was attempted through guest lectures and committee membership in various local, national, and international collaborative research committees and research working groups, such as the NIH working group on

Reproductive Endocrinology, the editorial boards of the Journal of the National Cancer Institute and Year Book of Cancer, the U.S.-Japan Medical Science program, and the International Commission for Protection against Environmental Mutagens and Carcinogens.

Significance to Biomedical Research and the Program of the Institute:

Epidemiologic surveys and detailed studies of families at high risk of cancer may help to detect environmental and genetic influences in carcinogenesis. In addition, identification of these families has therapeutic implications, enabling surveillance and early diagnosis of neoplasms and genetic counseling for offspring.

Proposed Course:

The same approach will be continued. New laboratory methods and epidemiologic clues from other sources will be incorporated into the project protocol as available.

Publications:

See Bibliography numbers: 1, 5, 6, 17, 19, 20, 24, 27, 31, 32, 33, 35, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 75.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04400-17 CEB

PERIOD COVERED
October 1, 1980 to October 1, 1981

TITLE OF PROJECT (80 characters or less)
Clinical Studies Section, Boston, Massachusetts

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Frederick P. Li Head, Clinical Studies Section CEB NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Clinical Epidemiology Branch, Field Studies and Statistics Program

SECTION
Clinical Studies Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.8	1.0	0.8

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Clinical Studies Section in Boston, Massachusetts is engaged in the identification of persons at high risk of cancer and investigations of causes of their susceptibility. The individuals are found through special referrals from clinicians, self-referral from patients, and clinical observations at Harvard-affiliated hospitals. With informed consent, epidemiologic studies are made to identify the predisposing role of cancer genes and environmental carcinogens. Laboratory studies are performed to clarify biologic mechanisms of susceptibility to cancer. Findings to date show that cancer risk approaches 100% in persons who are carriers of certain cancer genes. Early cancer detection has been achieved in some patients, and appropriate counseling during pre-clinical stages has been provided to other patients. Among high risk patients are those who had one cancer in childhood and are susceptible to develop multiple primary neoplasms. Risk factors in these patients are under study through the computerized Registry of Survivors of Childhood Cancer in Boston. These activities were shown during a 3-month period to an epidemiologist (Dr. Li Jun-yao) from the People's Republic of China as part of a fledgling binational exchange in cancer studies.

Project Description

Objectives:

1. To employ clinical observation at the bedside to identify causes of human cancers.

2. To investigate susceptibility factors in the development of cancers in high risk subgroups in the population.

3. To apply the latest laboratory techniques to investigate biologic mechanisms of predisposition to cancer.

4. To introduce methods of clinical epidemiology to investigators from the People's Republic of China and elsewhere.

Methods Employed:

Patients admitted for cancer therapy at the Sidney Farber Cancer Institute are examined for clues to etiology of the neoplasm. When exceptional clinical observations are made, appropriate follow-up epidemiologic and laboratory investigations are conducted.

A registry has been established of 820 patients who have survived childhood cancer for at least 5 years. These patients are being studied to determine the probability of development of a new cancer, and the somatic and genetic effects of the neoplasm in childhood.

Striking family aggregates of specific cancers have been identified. Family members are under study to identify reasons for the susceptibility, and to detect early cancers.

Prospective studies are made to confirm predicitions of high risk of cancers in individuals, families, and other groups.

Major Findings:

1. Follow-up studies of a family with the newly recognized genetic syndrome, ataxia-pancytopenia, confirmed the predicition that acute leukemia would develop in a child.

2. Prospective study of the familial breast cancer-sarcoma syndrome showed development of 16 cancers during a 12 year period, as compared with 0.5 cases expected. The new cancers were breast cancer or sarcoma in 9 instances.

3. Analysis of 820 survivors of childhood cancer shows an approximately 20-fold increased risk of development of multiple primary cancers. The new data are based on 8 years of prospective observation and confirm figures based previously on retrospective analyses.

4. Two brothers with familial polyposis coli and colon cancer developed acute leukemia, a new disease constellation. Their parents are first-cousins, suggesting that the condition is due to a recessive gene.

5. A patient with Turcot's syndrome (familial polposis coli and brain tumor) developed acute leukemia after cranial radiotherapy. Study of her fibroblasts suggests increased susceptibility to the effects of ionizing radiation. In vitro evidence of sensitivity to radiation was found in a second family in which 2 sisters with radiotherapy-treated Hodgkin's disease subsequently developed breast cancers within the treatment field.

6. Studies of long-term survivors of childhood cancer revealed the following new findings: 1) high risk of ovarian failure after radiation before puberty to both ovaries. No sterilizing effect of chemotherapy was detected in the study; 2) high frequency of thyroid dysfunction after radiotherapy in high doses. Elevated TSH was the major finding. Approximately 20% of 100 study patients had nodules, including carcinoma in 3 patients; 3) hign frequency of late effects, particularly kyphoscoliosis among patients treated in the first months of life; 4) small birth-weight infants born to women radiated for Wilm's tumor in childhood; 5) susceptibility to radiation-induced breast cancer after chest radiotherapy.

7. In collaboration with scientists from the People's Republic of China, the following observations were reported: 1) cancers of liver, nasopharynx and esophagus are unequally distributed within China; rates in Chinese-American migrants are under study; 2) childhood leukemia occurs in older children in China as compared with the U.S.; 3) Ewing's sarcoma seems to be rare among the Chinese; 4) high rate areas for penis cancer in China also show increased cervix cancer rates, suggesting a common etiology of the 2 neoplasms.

Significance to Biomedical Research and the Program of the Institute:

The Clinical Studies Section identifies persons susceptible to cancer for laboratory studies of mechanisms of carcinogenesis. In addition, specialized laboratory techniques are investigated as markers to identify persons for surveillance for cancer at early stages. Follow-up studies of survivors detect late effects of disease and therapy that may lead to modifications of therapy to reduce morbidity. The collaboration with Chinese scientists provides additional knowledge of the patterns of cancer worldwide and clues to causes of certain prevelant neoplasms in the U.S.

Proposed Course:

The Clinical Studies Section intends to continue studies of childhood cancers. These projects will examine the etiologic role of genetic factors and pre-natal exposures, and the late effects of these diseases. In addition, the methods that have proved useful in childhood cancer studies will be applied to study appropriate cancers in adults. These studies will examine family aggregates of cancer, and epidemiologic features of rare and seldom studied forms of adult malignancies. High risk persons will continue to be surveyed to detect cancer at treatable stages, and to receive counseling and supportive care.

Dr. Li has helped to arrange one-year visits to NCI by epidemiologists from the Shanghai Cancer Institute (Dr. Tu) and the National Cancer Institute in Beijing (Dr. Li Jun-yao). They are jointly engaged in descriptive studies of cancer in China. From descriptive data, plans for analytic studies will be developed, perhaps in binational projects.

Publications:

See Bibliography Numbers: 18, 21, 22, 28, 36, 37, 38, 40, 41, 42, 43, 44, 45, 46, 48, 76.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 05137-02 CEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Bedside Etiologic Consultative Program

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI :	Robert W. Miller	Chief	CEB	NCI
OTHER:	Priscilla A. Gilman	Expert	CEB	NCI
	Judith L. Bader	Clinical Investigator	CEB	NCI
	Elisabeth A. McKeen	Clinical Investigator	CEB	NCI
	Bessie Chen	Medical Student		

COOPERATING UNITS (if any) NCI wards; Children's Hospital National Medical Center, Washington, D.C.; Johns Hopkins Medical Institutions, Baltimore, Md.; University of Maryland Medical School, Baltimore, Md.; Georgetown University Medical Center, Washington, D.C.; University of California, San Francisco, California

LAB/BRANCH
Clinical Epidemiology Branch, Field Studies & Statistics Program

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.8	0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

By participation of the staff at area institutions, at the bedside and in clinical conferences, rounds and oncology clinics, patients or families with unusual features are ascertained and etiologic consultations given. Comprehensive family interviews and appropriate laboratory and epidemiologic investigations are suggested or performed yielding environmental and genetic information as a means of elucidating biologic mechanisms of carcinogenesis.

PHS-6040
(Rev. 2-81)

Project Description

Objectives:

1. To generate hypotheses as to etiology of human cancer from clinical observations made at the bedside.

2. To study these hypotheses through epidemiologic and laboratory investigations.

3. To identify pregestational and prenatal factors predisposing to malignancy, including genetic and environmental interactions.

4. To increase the sensitivity of primary and specialty care clinicians to clues of etiologic significance in their patients and guide them in appropriate evaluation.

Methods Employed:

At medical centers in Washington and Baltimore, Branch members participate regularly in case-review conferences, rounds, and clinics leading to identification of patients with unusual features and etiologic consultations are performed. Follow-up epidemiologic and laboratory studies are conducted at the parent institution or through the contract laboratories of CEB. At Georgetown University, questionnaires for family histories and environmental exposures are given to all pediatric and medical oncology patients as an initial screening device. More complete family interviews are included in the consultation. Two staff persons have academic appointments (Dr. Gilman, Lecturer in Pediatrics and Pediatric Oncology at Johns Hopkins, Dr. McKeen as Clinical Instructor in Medicine at Georgetown) which allow access to tumor registries and record-room research material for more complete epidemiologic data gathering.

Etiologic consultations are provided on request on the wards of NCI and in the Inter-Institute Human Genetics Clinic. Other requests for consultation come by mail or telephone, and during visits to lecture at hospitals or universities in other parts of the country. The Childhood Cancer Etiology Newsletter, issued monthly since December 1973 by the Branch to 900 recipients throughout the world, assists in this endeavor; as do the examples set by the Branch in its actions and publications.

Major Findings:

1. A single case seen on rounds at the Children's Hospital National Medical Center in D.C., noted by our staff member, led to the realization that pineal tumors occur excessively with bilateral retinoblastoma. We have since found 14 such cases, which suggest that the gene for retinoblastoma affects retinal anlage wherever it may occur naturally or ectopically.

2. Two patients at Georgetown University with renal cell carcinoma (RCC) were noted by our staff member to have accessory nipples. A case-central study of 32 RCC patients in this area revealed that 6 had accessory nipples as compared with 0.5 expected. Embryologic development of the breast and kidney may have a previously unrecognized link with the biology of renal

cancer. Further study may suggest a new concept in renal cell carcinogenesis.

3. Another patient with familial cancer, seen by our staff, has a chromosomal deletion which may indicate the locus for a gene that influences the development of the cancer.

4. Predisposition to malignancy in congenital bone marrow dysfunction disorders has been confirmed (3 acute leukemia and 1 histiocytic lymphoma in less than 75 cases of infantile genetic agranulocytosis and 4 acute leukemia and 2 liver cell carcinomas in less than 200 cases of congenital hypoplastic anemia). Epidemiologic and laboratory studies are underway to elicit the mechanism(s).

Significance to Biomedical Research and the Program of the Institute:

The regular participation of CEB staff in oncology activities at the area institutions is increasing the oncologists' awareness of and ability to identify unusual associations of etiologic significance. Referral to or more detailed investigations in these patients by epidemiologists and other laboratory investigators will allow identification of genetic, familial and/or environmental factors predisposing to malignancy. By subsequently identifying high-risk features or persons, prevention or early diagnosis of malignancy can be facilitated.

Proposed Course:

It would seem valuable to explore possibilities of a more formal collaboration between pediatric oncology divisions at the area institutions and the CEB to allow improved pediatric oncology registry data, continuity of studies, case ascertainment for further investigation, and teaching. Combination of material from the different locations by an epidemiologist could yield etiologic relationships more rapidly. Dissemination of information on genetic and prenatal factors would be easier, leading to earlier diagnosis or prevention.

Publications:

See Bibliography numbers: 3, 25, 26, 27, 32, 37, 49, 52, 59, 77

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 05139-02 CEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

NIH Inter-Institute Genetics Clinic

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I. :	D.M. Parry	Visiting Scientist	CEB	NCI
Other:	J.L. Bader	Clinical Investigator	CEB	NCI
	J.J. Mulvihill	Chief, Clinical Genetics Section	CEB	NCI
	P.A. Gilman	Expert Scientist	CEB	NCI
	E.A. McKeen	Clinical Investigator	CEB	NCI

COOPERATING UNITS (if any)
NIAMDD, NIH; NICHD, NIH; NEI, NIH; NINCDS, NIH; CC, NIH

LAB/BRANCH
Clinical Epidemiology Branch, Field Studies and Statistics Program

SECTION
Clinical Genetics Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) The Genetics Clinic is a collaborative undertaking by researchers from five different institutes and the Clinical Center. Consequently, Clinic patients constitute a broad spectrum of genetic disease. The patient load during the Clinic's second year comprised 300 individuals representing 60 different diagnostic categories. Of these, 97 patients (32%) were seen by members of CEB. For our Branch the Clinic provides a multidisciplinary setting in which to study unusual patients who either have cancer or increased risk of developing malignancy. Patients are ascertained through special referrals from outside physicians and inhouse requests for etiologic consultations. With informed consent, the approach to the patient includes detailed physical examination and, where applicable, epidemiologic studies of the environmental and genetic background and laboratory studies to clarify biologic mechanisms of carcinogenesis. Categories include patients with neurofibromatosis, a common autosomal dominant disorder that has an increased predisposition to cancer, patients with birth defects and cancer, families with childhood sarcomas and breast cancer in blood relatives, and any other families with an excessive occurrence of cancer of any type.

PHS-6040
(Rev. 2-81)

Project Description

Objectives:

1. To identify genetic and environmental factors in the development of human cancer.

2. To utilize the insights provided by the multidisciplinary approach of the Clinic to pursue new avenues of clinical and laboratory investigation into cancer risk.

3. To provide counseling for persons at high risk of malignancy and recommend appropriate medical surveillance for the early detection of tumors.

Methods Employed:

Referred patients are examined to determine the extent of any pre-existing condition or birth defects and for clues to the etiology of cancer in themselves or family members. When exceptional clinical observations are made, appropriate follow-up epidemiologic and laboratory investigations are conducted. Certain categories of patients, i.e., those with neurofibromatosis are examined and tested according to an established protocol to ensure uniform data collection.

Major Findings:

1. Palms and fingers have complex ridge patterns whose expression is determined by the interaction between multiple genes and environmental forces early in fetal development. Pattern specific alterations are known to occur in some genetic syndromes. Prints of palms and fingers have been obtained from a large number of individuals with neurofibromatosis and their unaffected first degree relatives. Preliminary analysis by the computer program of Dr. Nina Steg of the Du Pont Institute indicated that the NF patients seen by members of the Clinical Epidemiology Branch differed in their dermatoglyphic characteristics from those seen at the Du Pont Institute who have been ascertained primarily through orthopedic problems. We are now in the process of analyzing our data to see if any parameter or group of parameters will distinguish affected individuals from unaffected first degree relatives and controls.

2. A case control study of renal adenocarcinoma suggested an association between that cancer, accessory nipples and genitourinary anomalies. Individuals with renal cancer and such anomalies were further evaluated in the clinic. Two brothers with renal cancer, anomalies of the nipples and genitourinary tract and their sibs were examined for other anomalies which might be embryologically related. Also examined were two other individuals with renal cancer and a first degree relative with brain cancer. This

protocol included peripheral blood cell karyotypes, dermatoglyphics, X-rays and an extensive physical examination.

3. We are interested in delineating the genetic and phenotypic heterogeneity in patients with the Diamond-Blackfan syndrome (congenital hypoplastic anemia) which has been reported with leukemia and other malignancies. A 22-year-old white female, with Diamond-Blackfan syndrome and short stature was seen for evaluation in clinic. Her disease may be either sporadic or autosomal recessive. No other sibs are affected. She had no congenital anomalies, but bone marrow chromosomal studies were abnormal, with 10% breakage.

4. Members of a family were examined in which the proband, a 22-year-old white male, had a "cured" Wilms tumor and minor congenital anomalies. Two deceased sibs had dextrocardia with cyanotic congenital heart disease and a living examined sister had situs inversus, vertebral and ear anomalies. Special chromosome studies have been initiated to look for relevant cytologic abnormalities and the proband's sperm will be examined for abnormal ciliary motility, which, as a generalized defect, has been proposed to be responsible for situs inversus as seen in Karteganer's syndrome.

Significance to Biomedical Research and the Program of the Institute:

The Clinic provides a unique multidisciplinary setting in which unusual occurrences of cancer can be identified and studied by geneticists, epidemiologists and laboratory investigators. The regular post Clinic conferences and seminars provide major vehicles for dissemination of new findings in cancer etiology to scientists representing a broad array of clinical and laboratory expertise and offer opportunities to establish future insightful collaborations.

Proposed Course:

The Genetics Clinic will continue to provide a unique setting for the study of genetic and environmental factors predisposing to increased cancer susceptibility. In our effort to learn about the biologic causes of cancer we will continue to ascertain and study patients with genetic diseases predisposing to cancer, familial aggregates of cancer, and patients with birth defects or unusual environmental exposures associated with tumor development.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 05146-02 CEB

PERIOD COVERED October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Morbidity in Childhood Cancer Survivors and Their Offspring

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI's:	J.J. Mulvihill	Chief, Clinical Genetics Section	CEB	NCI
	M.H. Myers	Chief, Biometric Research and Analytic Studies Section	BB	NCI
Other:	M.R. Hanson	Epidemiologist	CEB	NCI
	M.J. Mitchell	Demographer	CEB	NCI
	S. Abbott	Statistician	BB	NCI
	R.R. Connelly	Statistician	BB	NCI
	E.A. McKeen	Clinical Investigator	CEB	NCI
	J. Blatt	Senior Associate	POB	NCI
	F. Cosner	Chief, Medicine	Queens Hospital, NYC	

COOPERATING UNITS (if any)

Biometry Branch, Field Studies and Statistics

LAB/BRANCH
Clinical Epidemiology Branch, Field Studies and Statistics Program

SECTION
Clinical Genetics Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.1	2.0	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Fertility and reproductive history in cancer patients, especially in long-term survivors of childhood cancer, and in men and women who reproduced during cancer therapy are studied for possible mutagenicity and teratogenicity of cancer treatments, and to discover hereditary patterns of cancer. Current phases include (1) interviews of 2,800 adults who survived cancer in childhood and their sibs as controls; (2) recall of National Cancer Institute patients for detailed examinations of offspring; and, (3) registry of pregnancies in young adult women through correspondence with physicians in Cancer and Acute Leukemia Group B.

PHS-6040
(Rev. 2-81)

Project Description

Objectives:

To document fertility and reproductive outcome in patients with cancer before, during, and after treatment. The goals are to test genetic theories of cancer etiology; to define potential gondal toxicity of cancer treatment, both teratogenicity and mutagenicity; and to provide needed information for genetic counseling of long-time survivors of cancer. The hypothesis being examined is that cancer patients, especially, have excessive morbidity due to additional malignancies or other illnesses and have impaired reprodutive performance, including an increased frequency of live offspring with birth defects or cancer.

Methods Employed:

Three separate phases are in different stages of completion.

1) A registry of young women with cancer was assembled from physicians of Cancer and Acute Leukemia Group B. Preliminary analysis of Hodgkin's disease patients, the single largest group was already published, and a comprehensive analysis of 237 pregnancies in 66 women is under way.

2) Recall of patients of the Clinical Oncology Program of the National Cancer Institute, especially the Pediatric Oncology Branch, was undertaken to permit personal examination of as many offspring as possible. Of 448 patients screened, 30 reported 12 abortions and 28 live births. No major malformation was seen, nor impairment of growth, development, or school performance. Included were 7 offspring exposed in utero to aggressive chemotherapy.

3) Intensive interviewing and record abstracting are underway among individuals in California, Connecticut, Iowa, Kansas, and Texas who had cancer under age 19 years, survived at least 5 years, and achieved at least age 18 years. The controls are up to two sibiings per case. The cases include 2,840 patients (26% lymphoma, 15% brain tumor, 11% soft tissue sarcoma, and 7% embryonal tumors) with a mean age diagnosis of 15 years, and a mean year of diagnosis of 1962. Treatment regimens were surgery only in 37%, radiation only in 20%, both in 17%, and some chemotherapy in another 17%.

Major Findings:

Approximately 10% of the total study population have been interviewed to date. Preliminary editing has been done on the first batches of data that have been keypunched. Due to governmental regulations, California has not yet begun full-scale interviewing. Personnel problems in Texas have delayed return of data.

Significance to Biomedical Research and the Program of the Institute:

The study of reproduction in cancer patients may help document the familiality of certain tumors (especially of childhood), the predicted but poorly documented teratogenecity of modern cancer therapy, and the predicted but undocumented germinal mutagenicity of radiation and drugs. The data may be directly used in counseling cancer patients.

Proposed Course:

The case registry of Cancer and Acute Leukemia Group B is closed, although comprehensive analysis continues. The staff of the Clinical Oncology Program continues surveillance of their patients. The five-center study is scheduled to end the collection of data by Fall, 1982.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 05194-01 CEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

National Cancer Mortality Studies by Computer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI :	R.W. Miller	Chief	CEB	NCI
OTHER :	F.W. McKay	Computer Systems Analyst	CEB	NCI
	M. Hanson	Epidemiologist	CEB	NCI

COOPERATING UNITS (if any)
National Center for Health Statistics
Bureau of the Census
DCRT

LAB/BRANCH
Clinical Epidemiology Branch, Field Studies & Statistics Program

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have used information from the National Center for Health Statistics (NCHS) and Bureau of the Census to create a comprehensive data base concerning mortality and population information at the county level. Data are available, 1950-1976, for cancer mortality, and 1965-76, for deaths from other causes. The data will be extended through 1978 when public use NCHS mortality tapes are received. Population data will be extended and corrected when the 1980 census data become available. Three-dimensional graphs employing these data are one example of the value of the data collection. A system for mapping counties in black-and-white is being developed, along with a system for analyzing projections of cancer mortality in the coming decades.

PHS-6040
(Rev. 2-81)

Project Description

Objectives:

1. To develop new ways for evaluating existing cancer mortality data for the United States by computer.

2. To project the numbers of cases expected in the next 20 years based on changes in the age distribution of the population; e.g., the baby boom of the 1950's.

3. To provide special data tabulations to others on request.

Methods Employed:

The data, which were collected by NCHS in a varying format from year to year, 1950-1977, have been reworked into a format in common. The widely known Atlases of Cancer Mortality in the U.S. by County were the first results from studying these data. Programs have now been developed for creating three-dimensional graphs of cancer mortality rates by site, color, sex, calendar year and age-group. The graphs are drawn on a Calcomp X-Y plotter.

The most efficient use of computer time has been achieved through the use of programs written in COBOL, Fortran or Assembler. Over 30 fast-running programs are used to keep the data-base current. Most programs are run often enough to keep their operation efficient.

Major Findings:

1. The three-dimensional graphs, which allow trends in cancer mortality to be seen in a glance, are almost ready for publication as a 500-page reference volume.

2. The corresponding graphs for the population indicate changes in numbers of cases to be expected as the age-composition of the nation changes over time.

3. Combining the two can provide estimates of the needs for services in cancer care in the future, taking into account the pattern of the rates (i.e., rising, falling or unchanging).

4. Other uses of the data during the year included:

    a. Created graphs, 1950-1977, by single year of age from birth to 17 years for leukemia and for total mortality.

    b. In response to a request, provided color mapping system to the Nuclear Regulatory Commission with data files that define the boundaries for the 3056 counties of the U.S.

    c. For an evaluation of the accuracy of death-certificate diagnoses by C. Percy (Biometry Branch), extracted mortality data for 1971 for certain states and counties.

d. Wrote a program to isolate a long-standing problem in the NIH version of an IBM computer program known as the American National Standard COBOB Compiler.

e. Developed a black-and-white computer-mapping capability that involves variable shading of rasters within a county. Further development should produce 3 or 4 shades of gray plus no shading.

f. Used data for selected counties in Arizona, California, Nevada and Utah for evaluation of cancer mortality in relation to nuclear fallout by the Nuclear Regulatory Commission.

g. To analyze 5-year calendar groupings of cancer and other mortality, in collaboration with Dr. Michael Greenberg of Rutgers University, we updated data through 1970-1975 to supplement the previously available cancer mortality pentads for 1950-1969.

h. Provided data for liver and biliary tumors in selected counties in Virginia to the Bureau of Kepone Studies in the State Health Department.

i. For the EPA, provided annual data, 1960-1965, and programs for evaluation to estimate the distribution by race, to compensate for omission of this risk factor in New Jersey's death-certificates in 1962 and 1963.

j. Edited and re-ordered a list of toxic chemical spills in transit (provided by DOT) so tabulations could be made for epidemiologic study by the Committee on Environmental Hazards of the American Academy of Pediatrics.

k. Provided data on mapping systems to the several university groups.

l. Provided computer services for an update of a study on bovine leukosis and human cancer originally published by Dr. W.A. Priester, Head of the CEB Veterinary Section. The statistical analysis is being performed by Dr. B.J. Stone of EEB.

m. Repeatedly corrected a multitude of typographical errors in preparing NCI Monograph 54 for publication. The many tables produced by our computer programs had been set in type under contract rather than photocopied. The volume, dated November 1980, was issued in March 1981.

Significance of Biomedical Research and the Program of the Institute:

The computer-generated volumes of tables and graphs of national cancer mortality are widely used, and special requests are frequently received and information provided.

Proposed Course:

Further work on county mortality graphs to pool data on adjacent areas of special interest, as, for example, from radioactive fallout or chemical contamination.

Publications:

See Bibliography numbers: 29, 30, 47, 83

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 05198-01 CEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Clinical Epidemiology of Neurofibromatosis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI :	Judith L. Bader	Clinical Investigator	CEB NCI
OTHER:	Nancy A. Strickman	Epidemiologist	CEB NCI
	Dilys M. Parry	Geneticist	CEB NCI

COOPERATING UNITS (if any) Clinical Center, NIH; National Institute of Arthritis, Metabolism and Digestive Diseases; National Eye Institute; National Institute of Neurological and Communicative Disorders and Stroke; National Neurofibromatosis Foundation; Late Effects Study Group

LAB/BRANCH
Clinical Epidemiology Branch, Field Studies & Statistics Program

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.75	0.25

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☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Individuals with the autosomal dominant genetic disorder, neurofibromatosis (NF), are thought to be at increased risk of cancer, particularly neural tumors. Multidisciplinary study of individuals with this condition was undertaken (1) to seek a reliable biomarker for NF, (2) to use genetic linkage analysis to localize the NF gene, (3) to quantify more precisely the frequency and pattern of cancers associated with NF, and (4) to evaluate selected patients and families with NF (e.g., twins, homozygotes) to learn about factors which influence the clinical heterogeneity of the condition and its link with cancer. Patients with this disorder were evaluated in the Inter-institute Genetics Clinic, and on field trips. In addition, data on affected individuals were provided by the National Neurofibromatosis Foundation and the Late Effects Study Group.

PHS-6040
(Rev. 2-81)

Project Description

Objectives:

1. To seek a reliable biomarker for NF.

2. To use genetic linkage analysis to localize the NF gene.

3. To quantify more precisely than before the frequency and pattern of cancers associated with NF.

4. To evaluate selected patients and families, (such as twins, homozygotes, skipped generations and persons with segmental disease) to learn about factors that influence the clinical heterogeneity of NF and its link with cancer.

5. To develop leads for new laboratory investigations into the diagnosis of the disease and the carcinogenic process.

6. To counsel patients and families and to recommend the appropriate medical surveillance for early detection of tumors.

Methods Employed:

Referred patients are examined at the Inter-institute Human Genetics Clinic to determine the stigmata of NF. Specimens are obtained with permission for studies which may lead to better diagnosis of patients with the NF gene who do not express its manifestations, as in skipped generations or persons with minimal disease. This is in connection with the search for a biomarker of the disease. Blood samples are obtained in appropriate families for studies of genetic linkage which may locate a nearby gene for NF. A standard questionnaire is completed for all new patients to determine the family history and to seek information concerning events which increase or decrease progression of the disease. The questionnaire has also been sent to all persons with NF who are members of the National Neurofibromatosis Association, to evaluate the nature of the disease, the occurrence of neoplasia in it and causes of death in a sample different from those that can be obtained in hospitals. Some of the respondents do not have severe enough disease to be hospitalized. With regard to children with NF, data from the Late Effects Study Group has been evaluated to determine how many children with cancer were noted on the hospital records also to have NF.

Major Findings:

1. The data from the Late Effects Study Group showed that neurofibromatosis is the most common single genetic disorder associated with childhood cancer, having been found in approximately 1% of all cases.

2. Identical 35-year-old female twins discordant for NF (one with and one without the disease) were examined, with special attention to evidence that they were indeed monozygous insofar as could be determined. An adequately documented occurrence of this type has not previously been reported. In the absence of a reliable biomarker we cannot determine if (a) the affected twin has NF but the gene is non-penetrant (a rare event) or (b) the genetic mutation occurred post-zygotically soon after twinning.

3. Because the clinical stigmata of NF are exacerbated during puberty, pregnancy, with exogenous estrogens, and possibly during menopause, we thought that estrogen receptors might play a role in this effect. Evaluation of estrogen receptors in five dermal neurofibromas and one neurofibrosarcoma were negative, however. Additional patients will be similarly evaluated.

4. Segmental neurofibromatosis (stigmata of NF confined to a localized area of the body) has been observed in 10 patients and is thought to represent a late post-zygotic somatic mutation. Offspring of patients with segmental NF have not previously been affected, but we have found a 42-year-old man with segmental NF whose offspring was affected.

5. In collaboration with M. Anne Spence, Ph.D., of the Division of Medical Genetics, University of California, Los Angeles, five families with multiple generations affected were evaluated for genetic linkage with polymorphic red or white cell markers. HLA A&B were found not to be linked to NF and the results concerning other markers are pending.

6. Evaluation of the frequency and pattern of cancer with NF was evaluated in 318 affected individuals from 156 families through data provided by the National Neurofibromatosis Foundation. Using an approach not previously applied in studies of NF with cancer, we showed that NF increases the risk of malignant neural tumors at least 18-fold in males and 35-fold in females.

7. Speech evaluation of all NF patients seen in our clinic revealed that over 90 percent had a variety of speech and oral motor deficits unrelated to hearing problems or structural lesions of the head and neck. Review of the literature failed to uncover any previous studies focusing on speech defects with NF. We suspect this is a significant but previously unrecognized common manifestation of NF.

8. Evaluation of dermatoglyphics in NF patients failed to confirm a previously reported distinctive hand-print pattern which distinguishes, by computer analysis, affected from unaffected individuals.

9. An 11-year-old girl with NF was identified who is the offspring of two affected parents. All three were evaluated in our clinic. Although the child is more severely affected than either parent, no laboratory method yet known can confirm that the child is a "double dominant" (i.e., a homozygote). The mother had two early first trimester spontaneous abortions, which might have been the consequence of NF homozygosity. No such incidence has yet been reported in the medical literature.

Significance to Biomedical Research and the Program of the Institute:

Although NF affects about 75,000 Americans and predisposes markedly to cancer, it has not previously been well studied. The predisposition to cancer offers possibilities for learning something new about the biology of neoplasia which may lead to improved prevention, detection or treatment not only for patients with NF, but for others in the general population with related cancers.

Proposed Course:

Individual patients of special etiologic interest will be evaluated in the clinic, such as those who over react to X-rays or drugs, or who have associated congenital anomalies which, if they are chromosomally induced, may indicate the locus of the gene for coexistent NF. Laboratory collaboration will continue in studies of biomarkers, genetic linkage, estrogen receptors, and cell survival of skin fibroblasts in culture after exposure to gamma radiation and certain chemicals. Analysis of data from the National Neurofibromatosis Foundation will evaluate an influence, if any, of paternal age on the course of the disease, on the fertility pattern in affected males vs. females, and in the definition of cancer risk.

Publications:

See Bibliography numbers: 23, 33, 74

CONTRACT NARRATIVES

CLINICAL EPIDEMIOLOGY BRANCH, DCCP

CONTRACT	TITLE	PAGE NO.
Atomic Energy of Canada, Ltd. (NO1-CP-81102)	*In Vitro* Radiosensitivity and DNA Repair in Genetic Syndromes and Families at High Risk of Malignancy	1104
Biotech Research Laboratories, Inc. (NO1-CP-61011)	Cytogenetic Evaluation of High Risk Cancer Families	1105

Clinical Genetics Section

ATOMIC ENERGY OF CANADA, LTD. (NO1-CP-81102)

Title: In Vitro Radiosensitivity and DNA Repair in Genetic Syndromes and Families at High Risk of Malignancy.

Contractor's Project Officer: Malcolm C. Paterson, Ph.D.

Project Officer (NCI): Elisabeth A. McKeen, M.D., Robert W. Miller, M.D.

Objective: To determine in vitro radiosensitivity and radiation induced DNA repair in high risk persons, e.g. members of cancer families and individuals with multiple primary tumors or genetic disorders predisposing to cancer.

Methods Employed: Using standard techniques, fibroblast cell cultures from high risk persons are established and assayed for mycoplasma contamination. An initial assay of radiosensitivity is performed measuring colony formation after in vitro irradiation; the results are compared with colony formation of control and ataxia-telangiectasia fibroblasts. On cell lines with abnormal radiosensitivity, further assessment of DNA repair is performed. Cells are also tested for sensitivity to radiomimetic compounds, N-Methyl-N-nitro-N-nitrosoguanidine (MNNG), ethyl nitrosourea (ENU), and 4-nitroquinoline 1-oxide (4NQO).

Major Findings: The colony formation assay identified 17 cell lines with abnormal responses to radiation and radiomimetic chemicals. Six (of six) cell lines from persons with hereditary cutaneous melanoma were sensitive to uv-radiation, but not γ-radiation; four were also sensitive to 4NQO. Fibroblasts from 6 affected members of a family with the Li-Fraumeni syndrome were abnormally resistant to radiation. Cell lines from affected female relatives of GM3314, a woman with Gardner's syndrome, were extremely sensitive to MNNG. A patient with bilateral retinoblastoma and pinealoblastoma also had in vitro sensitivity to γ-radiation. Work is in progress to identify the cellular mechanism of these abnormal findings.

Significance to Biomedical Research and the Program of the Institute: There is increasing evidence of the role of defective DNA repair in individuals with increased radiosensitivity and a redisposition to malignancy. Increased in vitro radiosensitivity and defective DNA repair has been demonstrated in fibroblasts of patients with the genetic disorders ataxia-telangiectasia, xeroderma pigmentosum, and familial retinoblastoma. This contract extends our investigation seeking new insight into the possible interplay between environmental and host-susceptibility factors by examining the mechanisms of DNA repair.

Proposed Course: The current project expires on June 3, 1981. A new contract will be awarded to expand this highly productive area of investigation. Fifty cell lines will be screened annually and abnormal specimens intensively investigated.

Date Current Contract Inititated: June 5, 1980.

Current Annual Level: 16,000

BIOTECH RESEARCH LABORATORIES, INC. (NO1-CP-61011)

Title: Cytogenetic evaluation of high risk cancer families.

Contractor's Project Officer: Shien Tsai, Ph.D.

Project Officer (NCI): John J. Mulvihill, M.D.

Objectives: To determine if persons prone to cancer because of their personal or family history have cytogenetic abnormalities.

Methods Employed: Standard karyotypic techniques, including differential staining for banding, are applied to biologic specimens from individuals at high risk for cancer under study by the Clinical and Environmental Epidemiology Branches. Specimens are lymphocytes from peripheral blood and fibroblast cultures. Dr. J. Whang-Peng, NCI, serves as consultants.

Major Findings: This year, 112 blood specimens and 3 tumor cell cultures were accessioned from members of 20 families with genetic or congenital disorders or an excess of cancer under study by the Epidemiology Branches. Major findings were:

1. For the first time, a constitutional chromosomal aneuploidy was associated with an adult tumor. Translocation between chromosomes 3 and 8 were found in all available tumor patients in a family in which 10 members in 3 generations had renal cell carcinoma.

2. Diverse manifestations of chromosomal imbalance were seen in a family with translocation between chromosomes 13 and 18: partial trisomy 18 syndrome, acute progranulocytic leukemia, gastric carcinoma, and, in carriers of the balanced translocation, azoospermia in males and ovarian cysts in females with relative infertility.

3. Confirmatory studies were made to verify the frequent finding of 11p- in children with Wilms' tumor and aniridia.

4. Additional preliminary observations concern: 1) high frequency of abnormal centromeres in 2 sisters with a new dysmorphic syndrome; 2) several "cancer families" with heterochromatic variants; and 3) several members of a family with normal lymphocyte karyotypes but mosaicism in fibroblast cultures.

5. Computer programs were written to search rapidly for statistically significant abnormalities in all results from this project.

Significance to Biomedical Research and the Programs of the Institute: The Epidemiology Branches search for genetic markers of human cancer by combining critical clinical and epidemiologic evaluation with a battery of modern laboratory approaches, one of which is provided by this contract. Several human malignancies are well known to be associated with cytogenetic abnormalities that may have etiologic, diagnostic, therapeutic, and prognostic implications. The contract provides a means for systematic cytogenetic investigations of persons or families at high risk of cancer.

Proposed Course: Specimens will stop being accessioned, except for critically or clinically important ones. Results will be interpreted in the context of our clinical-epidemiologic evaluation and other laboratory parameters of immunologic, genetic, and virologic nature. Contract will terminate within the year, which will be devoted to analysis of data in detail.

Date Current Contract Initiated: September 23, 1975.

Current Annual Level: $90,000.

ANNUAL REPORT
ENVIRONMENTAL EPIDEMIOLOGY BRANCH
October 1, 1980 THROUGH September 30, 1981

This is the sixth report of the Environmental Epidemiology Branch which was created in December 1975. The objective of the Branch is to generate and test ideas concerning the environmental and host determinants of cancer by a broad range of epidemiologic studies based on knowledge and application of clinical medicine and oncology, statistical methodology, new developments in carcinogenesis, and resources best available at the national level. Joining the Branch this year was Daniel Hoffman, Ph.D., who transferred from the PHS Bureau of Radiological Health. Appointed as Visiting Scientists were Dr. Li Jun-yao from the Cancer Institute of the Chinese Academy of Medical Sciences in Peking, and Dr. Elaine Ron from the Department of Clinical Epidemiology, Chaim-Sheba Medical Center, Tel-Hashomer, Israel. Appointed as Staff Fellows were Dr. Shelia K. Hoar (Sc.D. in epidemiology from Harvard University) and Dr. Deborah M. Winn (Ph.D. in epidemiology from the University of North Carolina). Elizabeth J. Martin received an Expert position to develop computer systems for large-scale epidemiologic studies.

RESEARCH PROGRAM

Geographic Studies

Demographic patterns--To provide a systematic means for identifying geographic variation and clustering, the Branch has analyzed U.S. cancer mortality on a county level and prepared computer-generated color maps for 35 cancer sites. First, an atlas of cancer mortality was published for the white population, followed by a companion atlas for the non-white population covering the years 1950-69. Maps for non-neoplastic diseases have also been prepared, emphasizing conditions that predispose to cancer or share etiologic factors. Mapping of the 1970-75 mortality from some of the more common cancers was conducted during this past year. Most striking perhaps was the updated map for lung cancer among white males, which revealed a shifting geographic pattern compared to the earlier period. Rates were high in broad stretches of the South, and elevated mortality was no longer seen in northern metropolitan centers.

Time trends over the 1950-75 period were examined for several cancers. Analyses by birth cohort showed that the rate of increase in lung cancer was considerably greater among blacks than whites. For males born in the late 1800s mortality in whites exceeded that in blacks by 50%, whereas for those born after 1915 the rates in blacks surpassed those in whites by 50%. Increases in mortality over time were also noted for non-Hodgkin's lymphoma, particularly the histiocytic type, for multiple myeloma, especially among blacks, and for malignant melanoma.

A survey of thyroid cancer in Connecticut showed increases in the papillary and follicular cell types, consistent with the effects of radiation therapy, given previously to children with benign conditions of the head and neck. A survey of Alaskan natives revealed an increasing incidence of "western" tumors (e.g., lung, breast, colon) which were uncommon in this population several decades ago. Attention was paid to a variety of other U.S.

population groups traditionally thought to have a deficiency of cancer overall, and on the high-rate cancers that nevertheless are seen in such "low-risk" groups. Although there was little fluctuation in the age-adjusted rates of mortality from breast cancer throughout the U.S. white female population, changes in time trends in age-specific rates were recorded and coincided with shifting patterns of childbearing in the first two-thirds of this century.

Field Studies--To seek explanations for the geographic and temporal variation in cancer across the U.S., correlation studies have related the county mortality rates with demographic and environmental data available at the county level. The clues derived from the cancer maps and correlation studies have been pursued actively by the Branch, the final step being the testing of specific hypotheses by case-control investigations in high-risk sections of the country. Risk factors for lung cancer were sought in southern coastal areas where the rates are highest in the U.S. In seaboard areas of Georgia and Virginia, earlier studies revealed significantly increased risks of lung cancer associated with employment in shipyards that operated during World War II. This year an interview survey in collaboration with the University of Miami was completed in Duval county (Jacksonville) Florida, which ranked first in lung cancer mortality among white males during 1970-75 among all urban counties in the country. Shipyard employment again contributed to the high rates, and a synergistic interaction between shipyard exposures and smoking was consistent with previous observations on asbestos workers. Asbestos exposures were implicated also in the increased risk found among construction workers. Excess risks were also associated with the lumber, forestry and fishing industries, but occupational factors could not fully account for the area's exceptionally high lung cancer rates. A small case-control survey of lung cancer in Bath, Maine, site of the oldest shipbuilding company in the U.S., brought to four the number of Branch interview studies showing an increased risk associated with employment in shipyards. When all data sets were combined, the relative risk for shipyard employment, adjusted for cigarette smoking, was 1.4, suggesting that as many as 100,000 extra lung cancer deaths may eventually result among the cohort of 4.5 million Americans involved in wartime shipbuilding activities.

Respiratory cancer is also the focus of case-control interview surveys in New Jersey with the State Department of Health, in coastal Texas with the University of Texas School of Public Health, and in southern Louisiana with the EPA and Louisiana State University. The latter study includes pancreas and stomach cancers, which also cluster in this area. Near completion is a case-control interview investigation of lung cancer in a tri-county area of eastern Pennsylvania, where a large nonferrous metal smelter is located. The interview data will be linked with environmental measurements of arsenic previously collected by other agencies in the area.

Among the most interesting of the cancer maps is that for cancer of the mouth and throat. Among males rates are high in urban areas of the northeast, while among females elevated mortality prevails throughout the South in rural as well as urban counties. A case-control study in collaboration with the University of North Carolina revealed that the southern excess was due primarily to the use of snuff, taken orally by over 40% of the women with oral and pharyngeal cancer. For cancers of the cheek and gum, where the tobacco powder is usually placed, the relative risk rose to nearly 50 among long-term

snuff users. Among non-users of snuff, cigarette smoking and alcohol consumption were the major risk factors and showed a synergistic interaction. No increased risk was found for employment in the apparel or textile industries, an association hypothesized by a previous correlation study and by smaller case-control studies in Britain.

Heavy alcohol consumption was the dominant risk factor in a case-control survey of esophageal cancer among black men in Washington, D. C., where the mortality rate from this highly fatal cancer exceeds the rates in all other U.S. cities, being higher than the national level for nonwhite males by 2.5-fold and for white males by 7-fold. Nutritional deficiency was also found to play an independent role, with decreased intake of fruits and vegetables, fresh meats, and dairy products.

A large-scale case-control interview study of bladder cancer in several parts of the country (descibed below) includes some high-risk areas, especially in males, and some low-risk areas. A separate investigation of bladder cancer is underway in rural New England, where the rates are elevated in both sexes. To clarify the relationship between woodworking and nasal cancer, a case-control interview study is being conducted in Virginia and North Carolina. To help explain the clustering of renal cancer in the north central area, the Branch has undertaken a case-control study with the University of Minnesota. Nearly 600 kidney cancer patients and twice as many controls were interviewed during the year. Also begun was a pilot study to examine reasons for the low rates, even at older ages, of colorectal cancer in retirement areas of Florida. This is provocative since most residents have migrated from high-risk areas of the north. If a selective migration effect can be ruled out, an in-depth interview and laboratory study will be initiated to probe for protective (nutritional?) factors in the southern environment.

Occupational Studies

Occupational studies have long played an important role in identifying environmental carcinogens. Many agents studied in workplace settings may also be found in air, water, food, or consumer products, so that occupational studies are invaluable in identifying and evaluating risks to the general population. They are initiated in order to (a) explain "hot spots" identified by county-by-county surveys of cancer mortality, (b) identify high-risk subgroups within broad industrial categories, (c) pursue clues provided by animal bioassays or clinical observations, (d) assist outside agencies or institutions in evaluating the health experience of their workers; and (e) provide insights into the basic mechanisms of carcinogenesis. Various approaches are employed utilizing data resources from industrial firms, labor unions, professional associations, and other government agencies.

The association between employment in the petroleum industry and cancer mortality, particularly cancer of the brain, has been under intensive investigation. Earlier Branch reports suggested that active workers in the petroleum refining and petrochemical industry experienced high mortality from leukemia and multiple myeloma and cancers of the brain, stomach, and kidney. A recent study of retired workers showed a similar pattern. The relative freqencies for leukemia, multiple myeloma, and non-Hodgkin's lymphoma were significantly

elevated and the number of deaths from brain cancer was slightly higher than expected. A proportionate mortality study of other petrochemical plants also revealed an excess of deaths from cancers of the brain and skin. Case-control studies using work histories from personnel records were initiated in several petroleum refineries to clarify these associations. Although the numbers are small, preliminary results indicated a larger percentage of brain cancer cases than controls in jobs that require movement and storage of crude oil and refinery products. In addition, the mean length of employment in occupations in the motor oil category (refinery operations producing lubricating oils, paraffin, and solvents) was much longer for cases than controls. The rubber industry is another area where solvents and complex hydrocarbons are used, but a case-control study of brain cancer in this industry revealed no increased risk for any of the occupational categories evaluated.

The development of nasal cancer in laboratory animals exposed to formaldehyde has raised concern that this chemical may be carcinogenic in humans. To evaluate this potential hazard, a proportionate mortality study of embalmers licensed by the state of New York was undertaken. Significantly more deaths from skin cancer were observed than expected, particularly among those first licensed before age 30 or first licensed more than 35 years ago. In addition, the frequencies of death from cancers of the kidney and brain were also slightly increased. There was no excess mortality from nasal or other cancers of the respiratory tract. Mortality studies of other occupational groups having contact with formaldehyde have been initiated.

A preliminary investigation of mortality among professional artists suggested that people in contact with commonly used art materials (i.e., pigments and dyes, solvents, and metals) may be at increased risk of certain cancers. Male artists had more deaths from leukemia and cancers of the bladder, kidney, brain, prostate, colon, and rectum than did males in the general population. The excesses of leukemia and bladder cancer were particularly striking among painters, while cancers of the colon and prostate were significantly elevated among sculptors. Female artists, primarily painters, had an increased mortality from cancers of the rectum, lung, and breast.

It has been reported that farmers are at higher risk than other occupations for certain cancers, notably leukemia and lymphoma. Death certificates were used in a case-control approach to evaluate the leukemia and lymphoma experience of Wisconsin farmers. For leukemia, increased risks were noted among farmers born more recently, dying at younger ages, or residing in counties where fertilizer usage and dairy production were heavy. Elevated risks were also noted for non-Hodgkin's lymphoma. A population-based case-control study of leukemia and lymphoma is underway in Minnesota and Iowa to identify agricultural factors that may be involved in the origin of these tumors.

In a cohort study of male chemists employed at a chemical company, the total number of cancer cases and deaths were lower than expected. The risks for melanoma and cancers of the colon and prostate were slightly elevated, but a higher mortality from lymphoma and cancer of the pancreas, as previously reported among chemists, was not seen. A cohort mortality study of members of the American Chemical Society, now underway, should help clarify the cancer

patterns in this group. Deaths from lung cancer were found to be excessive among members of the International Molders and Allied Workers Union. The risk was most evident among workers in iron foundries, particularly among those dying before age 65.

Special attention was given this year to reviewing methodologic issues of risk assessment from occupational studies; evaluating occupational exposure classification systems and developing occupation and exposure linkage systems; and comparing physical and sociodemographic characteristics of smokers, exsmokers, and nonsmokers. For several major investigations the results are not yet available. These include studies of aerial and structural pesticide applicators, jewelry manufacturers, fur dyers, dry cleaners, formaldehyde producers and users, furniture workers, leather tanners and shoe manufacturers, aircraft mechanics, medical technologists, histology technicians, taconite miners, potters, farmers, stainless steel welders, plumbers, marine inspectors, and shipyard workers.

Radiation Studies

Studies of populations exposed to ionizing radiation and certain types of non-ionizing radiation are being conducted to investigate further the relationship between cancer risk and exposure to high doses and to improve estimates of risks associated with lower doses. An immediate practical need is for risk estimates on which to base regulatory and other decisions about the use of nuclear and radiological technology in medicine and industry, and to assess the value of exposure avoidance as a means of cancer prevention. The study of radiation-induced cancer is also a promising approach to understanding carcinogenesis in general.

To characterize the risk of radiogenic breast cancer, the three major sets of human data were analyzed in similar fashion. The exposed populations included survivors of the Hiroshima and Nagasaki atomic bombs, tuberculosis patients exposed to multiple chest fluoroscopies in Massachusetts, and post-partum mastitis patients treated with X-ray in Rochester, New York. The findings suggest the following: the risk of breast cancer is greatest in persons exposed as adolescents; the dose-effect relationship is consistent with linearity; fractionation does not appear to diminish risk nor does time since exposure; the interval between exposure and clinical appearance of radiogenic breast cancer is mediated by age-related factors (hormonal?) but is unrelated to dose.

A second followup of women who received multiple chest fluoroscopies in conjunction with pneumothorax treatment of tuberculosis in Massachusetts was conducted by mail questionnaire. The results reaffirm that repeated relatively low radiation doses pose a future risk of breast cancer, the risk may be cumulative, and a woman's lifetime risk of breast cancer is likely determined in part during early adult life. The estimation of radiation doses to body organs was refined, in collaboration with the Bureau of Radiological Health (FDA), and a mortality analysis performed. No excess mortality from total cancer, or from leukemia, lymphoma, or lung cancer was seen among fluoroscopically examined women.

A 10-year international radiation study of 30,000 cervical cancer patients, treated in 9 countries and followed clinically with blood studies, failed to observe an excess of leukemia, although the bone-marrow doses were large. The absence of an effect suggests that (a) high doses to small volumes of bone marrow may cause substantial cell-killing and minimal cell transformation; (b) patients may not have been followed long enough to observe an effect; or (c) women may be less sensitive than men to radiogenic leukemia.

Among 6,000 women with cervical cancer treated with radiation in Connecticut, a 40% excess of second primary cancers was observed (449 vs 313), primarily resulting from cancers of the bladder, kidney, rectum, corpus uteri, and ovary occurring 15 or more years after radiotherapy. A deficit of breast cancer was observed, possibly due to radiation-induced menopause or reproductive factors (e.g., early first pregnancy) that may be protective. This study has been expanded to include 20 other cancer registries around the world. Preliminary cohort analyses have been completed, and case-control studies are being conducted to quantify the risk of second tumors associated with radiation doses.

Using the resources of the Radiation Effects Research Foundation (Japan), an evaluation of pathological materials indicated that radiation-related breast cancers are morphologically similar to other breast cancers occurring in women of comparable age. Mortality from infectious diseases among A-bomb survivors during the periods 1946-50 and 1950-66 was unrelated to radiation dose, thus failing to confirm speculation that the A-bomb survivor studies might be biased because of differential mortality resulting from radiation-induced immune deficiencies. Since excess cancer risks are much greater in Hiroshima than Nagasaki, it would seem that neutrons are more potent carcinogens than gamma rays. New determinations of radiation doses, however, suggest little difference between cities in the amount of neutrons received and, if true, will require a reappraisal of the city differences in cancer risks. To evaluate the possible interaction between radiation and host factors that increase breast cancer risk (e.g., positive family history), a case-control study has continued in Japan.

In a study of 1,005 women treated with radioactive iodine and 2,141 women treated with surgery for hyperthyroidism at the Mayo Clinic, no increased cancer mortality or incidence was observed in those treated with 131-I. An overall excess mortality may have resulted from a selection bias where women with a poor survival expectation received 131-I therapy instead of surgery. There was a significantly increased incidence of cancers of the thyroid and other organs with high 131-I exposure, although the numbers of cases were relatively small. These results have prompted an extended follow-up of a larger PHS thyrotoxicosis study of 25,000 patients, in conjunction with the Bureau of Radiological Health.

A study to evaluate the risk of head and neck malignancies among children irradiated for enlarged tonsils is continuing. Physical examinations will be conducted on both irradiated and surgical patients to more accurately determine the risk of thyroid nodular disease. Blood studies will help detect radiation effects on the parathyroid and thyroid; chromosome aberrations in circulating lymphocytes will also be evaluated. A study of thyroid incidence

in birth cohorts in Connecticut found the pattern to coincide with the widespread use of radiation for benign head and neck conditions between 1920 and 1959. A case-control interview study of 200 thyroid cancer cases and 400 population controls in Connecticut was initiated to evaluate the influence of radiation and other risk factors.

A collaborative study of 10,000 children irradiated for ringworm of the scalp in Israel revealed excess risks of brain tumors, plus evidence of long-term mental and psychological damage. A significant risk of leukemia was observed in 515 patients with non-Hodgkin's lymphoma treated with total body radiation. The Surveillance, Epidemiology and End Results (SEER) registries are being used to identify patients treated for various cancers to evaluate the carcinogenic effects of radiation therapy and chemotherapeutic agents. A case-control study of over 200 children who developed second primary cancer following treatment for childhood cancer is continuing at 13 childhood cancer centers in six countries. A feasibility study of late effects in X-ray technologists was successful, involving 170,000 occupationally exposed workers. A case-control study of approximately 2000 cases of leukemia was started to evaluate the risk of lifetime diagnostic X-ray exposure, using the resources of prepaid health plans. Record linkage of data tapes in the Connecticut Tumor and Twin Registries was initiated to evaluate the association of childhood cancer and prenatal X-ray exposures.

Medicinal Agents

Studies of drug-induced cancer have been valuable in the discovery of new carcinogenic hazards and in the development of insights into mechanisms of carcinogenesis. This has been so, not because of the presence of a large burden of drug-induced cancer in our society, but rather because drug exposure usually involves high doses which can be assessed by standard epidemiologic approaches. Also, with the introduction of a variety of new drugs, and the increased utilization of existing agents for a wide range of therapeutic indications, there is increased concern about a potential expansion of the proportion of cancer produced by medications. The Branch has conducted case-control and cohort evaluations of several medicinal agents, which have come under suspicion because of clinical observations, laboratory experiments, demographic surveys, or simply a high index of concern because of widespread usage by relatively healthy individuals.

Several studies were undertaken this year to evaluate the carcinogenic effects of estrogenic compounds. In two case-control studies of breast cancer, involving over 1,000 cases and 1,000 controls, there was an excess risk associated with long-term use of menopausal estrogens. The excess was most prominent among women with a prior oophorectomy. High risks were also seen among women who used hormones in the presence of other risk factors, particularly a family history of breast cancer.

Another problem under study is the risk of developing cancer among patients treated with immunosuppressive and cytotoxic agents. The use of high-dose immunosuppressive drugs by kidney transplant recipients was associated with a 25-fold increased risk of lymphoma, and lesser excesses of lung cancer, bladder cancer, soft tissue sarcomas, cancers of the liver and bile ducts, and malignant melanoma. Several studies were completed on patients treated with cytotoxic drugs, utilizing various clinical trials in

collaboration with the NCI Division of Cancer Treatment. These studies have revealed an excess risk of acute leukemia associated with the use of alkylating agents. The risk appears to follow a dose-response relationship rising to several hundred times that expected among those receiving the highest doses, and is unrelated to the primary condition for which treatment was initiated.

Recent concerns have been raised about the potential carcinogenicity of certain antihypertensive agents, thyroid hormone supplements, diazepam (valium), phenothiazines, dapsone, and isoniazid. Evaluations of thyroid hormones, diazepam, and isoniazid have thus far revealed no excess risk of malignancy. The follow-upof patients treated with isoniazid indicated excess deaths from liver cirrhosis, suggesting that chronic as well as acute liver disease may complicate this treatment.

Nutritional Studies

This year the Branch expanded its research to clarify dietary factors in cancer etiology. Several of the case-control studies in high-risk areas have probed for nutritional exposures. In Washington, D. C., the U.S. metropolitan area with the highest mortality rate for esophageal cancer among black males, a case-control interview study revealed that poor nutrition, measured in various ways, and heavy alcohol consumption were the major risk factors. The risk due to poor nutrition was independent of alcohol consumption, and the least nourished third of the study population had about twice the risk of the most nourished third. In addition, in two rural counties of Nebraska with a cluster of colon cancer, the risk was primarily among people of Czech ancestry, and seemed associated with a high intake of fat and dairy products. A case-control study using death records has been designed to evaluate the low rates of colorectal cancer in the southern United States, including retirement areas with a large percentage of migrants from the North. It is suspected that dietary changes following migration may exert a protective effect against the development of these neoplasms. Dietary influences on breast cancer were also suggested by the higher beef and pork consumption found among patients in a case-control study in Alberta, Canada, conducted in collaboration with the Cross Cancer Institute. The findings may provide some support for the notion that high dietary fat is a risk factor for breast cancer.

A detailed dietary questionnaire has been incorporated into case-control studies of lung cancer in New Jersey to clarify the hypothesis that low vitamin A intake increases the risk of this cancer. The study will also examine interactions between dietary and other risk factors such as smoking and occupational exposure.

With HANES I, the first health and nutrition survey of the United States, 1971-74, the National Center for Health Statistics assessed the nutritional status of 23,000 representative American adults. The Branch is searching for geographic correlations between dietary and biochemical data from this study and the county rates for various cancers. The data are also being utilized for methodological studies of nutritional epidemiology. In cooperation with other Institutes and the NCHS, the Branch is preparing to follow up all the adults examined in HANES I, in an effort to relate dietary habits with the subsequent risk of cancer.

Case-Control Studies

This year the Branch conducted several case-control studies which were broad in scope and not limited to high-risk communities or to particular risk factors (e.g., medicinal agents, radiation). Analyses are being carried out on data from a large population-based interview study of bladder cancer involving 4000 patients and 7000 controls to assess risks associated with artificial sweetener use, coffee drinking, hair dye use, work in the chemical and leather industries, cigarette smoking, drinking water quality, and urinary tract infection. The preliminary findings suggested no overall association with artificial sweeteners, but excess risks were seen among persons who smoked heavily or who had a low baseline risk (i.e., female non-smokers unexposed to occupational hazards). In addition, there was no obvious increased risk with the use of hair dyes, a diminished risk associated with never having drunk coffee (but no dose-response relationship among coffee-drinkers), and a 2-fold risk associated with multiple urinary tract infections. Additional studies of bladder cancer are planned, including a nationwide sample of young patients to evaluate the influence of in-utero exposures to artificial sweeteners and other possible risk factors, an extension of the case-control study in Atlanta to permit a thorough evaluation of textile industry exposures, and a study designed to evaluate reasons for the high bladder cancer mortality rates among both sexes in rural areas of New England.

Some studies were conducted in collaboration with epidemiologists at Oxford University. A study of benign breast disease indicated a relationship with only a few of the risk factors previously identified for breast cancer. Oral contraceptive use, particularly long-term use, was associated with a decreased risk of fibroadenoma, chronic cystic disease, and non-biopsied breast masses. This apparent protective effect was confined to current users of the medication; those who had stopped more than one year prior to diagnosis showed no decreased risk. The reduction in risk also seemed related to the use of pills with a high progestogen content. In addition, an in-depth evaluation of non-invasive cervical abnormalities (i.e., dysplasia and in situ carcinoma) indicated that the previously noted relation to age at first intercourse may be explained entirely by the correlated association with number of different sexual partners. An increased risk was also suggested among smokers and users of oral contraceptives. In another study, utilizing data from the Oxford survey of childhood cancer, no association was found with cleft lip or palate, or with the occurrence of chickenpox during pregnancy.

The field interviewing phase for several case-control studies was completed, and analysis has begun. The studies include an evaluation of prenatal and early life risk factors among 300 young adults (age 18 to 40) with testicular cancer plus controls, an evaluation of endogenous and exogenous hormonal factors among 350 women with ovarian cancer plus controls, a study of 300 patients plus controls to identify risk factors for cutaneous T-cell lymphomas, and an interview study of over 400 patients with intraocular melanoma plus controls. In addition, data collection is underway for patients with multiple myeloma who are participating in the Acute Leukemia Group B, plus sibling controls, to elicit clues to environmental and host factors.

Family Studies

Epidemiologic patterns of cancer have suggested to many observers that the environment contributes much more to cancer risk than do genetic factors. It is more likely that inherited susceptibility and interactions with environmental influences are too subtle or complex for detection by ordinary epidemiologic means. On occasion, when susceptibility is conspicuous, as in cancer-prone families, research opportunities are provided that may clarify the role of genetic and environmental factors in carcinogenesis. Studies on high-risk families are conducted jointly with the Clinical Epidemiology Branch and with clinical and laboratory scientists at NIH and elsewhere.

The development of an integrated manual and computerized record-keeping system provides a framework for an expanding data base that grew by over 900 new families in the past year, bringing the total to more than 1,900 families. Utilizing this capability, sophisticated statistical genetic approaches were applied in the analysis of the relationship of autoimmune disorders and the familial occurrence of neoplasia, segregation and linkage analyses of melanoma-prone families, and linkage analysis of HLA to lymphoproliferative malignancy and autoimmunity in a family.

Interdisciplinary studies of high-risk families continue to provide new insights into the mechanisms of host susceptibility to cancer. This research strategy is illustrated by the evaluation of familial melanoma and its relation to the dysplastic nevus syndrome (DNS), a project underway over the past six years. Based on a detailed study of 400 members of 14 melanoma-prone families, the clinical and pathologic features of DNS have been precisely defined and educational videotapes for family members, clinicians, and pathologists disseminated. Thirty-one new primary melanomas were identified in study participants, documenting the value of close surveillance in the detection of early surgically-curable lesions. The role of these dysplastic precursors in non-familial melanoma was also established. *In vitro* ultraviolet (UV) radiation sensitivity in cultured skin fibroblasts of patients with hereditary melanoma and DNS suggests a biologic basis for host-environmental interactions in this syndrome. Since genetic analysis indicates that hereditary melanoma segregates as an autosomal dominant trait, a heritable enzymatic defect predisposing to melanoma, manifested as UV sensitivity, will be evaluated in informative kindreds. A new syndrome of heritable retinoblastoma, cutaneous melanoma, dysplastic nevi, and predisposition to sarcomas has been identified. Study of mechansims of carcinogenesis in family members may help clarify the relationship of UV and gamma-radiation repair defects.

A survey of 20 kindreds prone to diverse neoplasms, including bony and soft-tissue sarcomas, breast and brain cancer, leukemia, and other tumors is nearing completion. Most of the 183 neoplasms have been reviewed by NIH pathologists, and often found to be of rare types and unusual histology. *In vitro* study of fibroblast cells from seven members of one kindred showed that affected and high-risk individuals, but not spouses, are resistant to cell killing by gamma radiation. Biochemical characterization of this novel phenotype has important implications for understanding mechanisms of carcinogenesis, especially since family members appear unusually susceptible to carcinogenic exposures, including radiation.

Sophisticated immunogenetic studies in families prone to various lymphoproliferative disorders have identified the first link of an HLA-MT antigen to Hodgkin's disease susceptiblity (familial and sporadic cases), homozygosity for rare HLA-Dr antigens in familial acute lymphocytic leukemia, genetic linkage of Waldenstrom's macroglobulinemia and autoimmunity to HLA in a family, and HLA identity of sibs with hairy cell leukemia in the first reported familial aggregation of this disorder. New laboratory approaches, such as fluorescence activated cell sorter analysis and recombinant DNA gene cloning technology, are being applied to the evaluation of families prone to chronic lymphocytic leukemia where shared cell surface immunoglobulin determinants have been identified. A review of lymphoreticular neoplasia in close relatives of 21 mycosis fungoides probands revealed an excess of Hodgkin's disease, suggesting that genetically determined immunoregulatory defects may predispose to a variety of familial lymphoid tumors.

Clinical observations prompted a case-control study of renal carcinoma that identified an excess of polymastia and genitourinary abnormalities in patients and their close relatives. Cytogenetic study in one family suggested a terminal deletion of the long arm of chromosome 8 in affected brothers. Familial occurrences of testicular cancer were associated with a high incidence of urogenital anomalies. Families prone to bladder cancer and to ovarian cancer are under investigation in an effort to identify metabolic phenotypes accounting for the genetic predisposition.

Studies were made of a family with an inborn cytogenetic abnormality (13:18 translocation). Acute leukemia and stomach cancer occurred among the carriers, and defective gonadal function was suggested by the presence of infertility in family members, along with ovarian cysts and teratomas. In collaboration with the Sloan-Kettering Institute, studies were carried out on families prone to colon cancer. An abnormality of actin cable structure was found in fibroblasts of patients with familial polyposis, but not in colon cancer-prone families without polyposis. In both types of families, there was a growth abnormality of colonic mucosa, signaling an early mutational event. A patient with male breast cancer from a family prone to diverse malignancies showed in vitro sensitivity to ionizing radiation and bleomycin. Thymic irradiation as a child may have contributed to the development of this patient's tumor. A repository of biologic specimens on high-risk families remains a valuable source of materials for experimentalists investigating susceptibility mechanisms in carcinogenesis.

Infectious Agents

Viruses have not been causally tied to the origins of any human cancer, but the search continues on several fronts. One Branch member has focussed on African Burkitt's lymphoma (BL), which has been associated with infection by the Epstein-Barr virus (EBV) and perhaps malaria. In collaboration with the University of Ghana Medical School, a survey of BL was conducted from 1966-78. In the face of an overall decline in incidence, more patients presented with abdominal tumors and a clinical pattern that resembled what is seen in low-incidence areas. The relationship of malaria to EBV was explored in a cross-sectional survey. Malaria, a known immunosuppressant, had no effect on response to EBV infection; if both agents are important in the etiology of BL,

they probably operate independently. As measured by antibody and parasitemia, malaria was found to be more common in rural than urban areas, in accord with the distribution of BL.

High antibody to EBV has been reported to be a marker of increased risk of developing BL in African children. In Ghana, as elsewhere, males showed a 2-fold excess of BL, but females had higher titers to EBV than males at every age. Thus, whatever factor promotes the excess risk in males appears independent of EBV response. In other studies, the EBV titers did not change in BL patients who survived many months (up to 6 months) in remission before relapsing, so this is not a useful means of predicting relapse. In progress are chromosomal and HLA studies of BL in an effort to clarify pathogenic mechanisms.

In addition, using a serum bank established in the 1960s, the Branch is planning a seroepidemiologic study to evaluate the effect of herpes virus type 2 on the risk of carcinoma of the cervix.

Immunoepidemiology

Several studies are underway to identify and evaluate populations with altered immune states and unusual rates of malignancy. The risks of cancer of different sites are quantified for various groups of patients, and the characteristics and determinants of unusual risks are sought. The populations studied include renal transplant recipients, patients with inherited and acquired immune deficiency conditions, and groups of immunostimulated persons (e.g., hyperimmunized states, autoimmune diseases). Detailed analysis of renal transplant recipients indicated a 25-fold increased risk of lymphoma which appears within a year of transplantation, tends to arise in the central nervous system, is greater among recipients of cadaver than sibling grafts, and is more prominent among recipients in the earlier years of transplantation compared to recent periods. The risk of other cancers was increased two-fold, but affected only certain neoplasms as described above for immunosuppressive drugs. In collaboration with the University of Minnesota, a registry of cancers occurring in patients with genetic immunodeficiency diseases has revealed a pattern of cancer risk that resembles that noted among transplant recipients. On-going follow-up studies of leprosy patients, persons "hyper-immunized" by repeated vaccinations, and other groups of acquired diseases and procedures that alter immune status should help to clarify the immunologic mechanisms involved in carcinogenesis.

Methodologic Studies

Several Branch members contributed to the adaptation and development of statistical methods useful in epidemiologic studies. Wide use in the field required the second printing of a general text which features a library of programs for epidemiologic analysis using a programmable calculator. A new method based on a recursion formula was developed which allows rapid computation of exact conditional likelihood estimates of the relative risk in matched case-control studies regardless of sample size, greatly extending the applicability of this useful technique. The extent to which unconditional logistic analyses overestimate odds ratios from matched data sets was examined in a simulation study, and the conservative bias of the confounder score was

evaluated in other reports. The comparability of information from surrogate respondents in case-control interview studies was assessed. One report on statistical methods for cohort studies presented an alternate means of analysis of failure time data by the Cox proportional hazards model. Several expository articles were written about problems of estimating cancer risk from low doses of ionizing radiation, including the effect on statistical power of using general models reflecting the current state of knowledge about mechanisms of radiation carcinogenesis. The methodologic issues in descriptive and correlational studies, in low-dose risk estimation, in familial aggregations of cancer, and in the computation of multivariate statistics were also reported during the year.

Reviews

The Branch continued to provide comprehensive and critical reviews of etiologic factors in cancer. One staff member served as co-editor of a reference volume on the epidemiology and prevention of cancer. This year reviews were prepared on several cancers, including the lung, pancreas, biliary tract, multiple myeloma, non-Hodgkin's lymphoma, bone cancer, soft-tissue sarcoma, and non-melanoma skin cancer. The epidemiologic evidence for environmental carcinogenesis and genetic susceptibility was considered in detail. Particular environmental hazards were reviewed, including ultraviolet radiation, medications, occupational exposures, parasites, water pollution, and food additives. Several review papers concerning the health effects following exposure to ionizing radiation were written, including a general overview, a review of cancers following medical irradiation, the effect of radiation on the immune system, the statistical aspects of estimating cancer risks from low doses of ionizing radiation, the epidemiologic issues concerning low-dose radiation studies, the implications of studies on radiogenic breast cancer for models of human carcinogenesis, and the long-term effects of radiation upon the human fetus. Also reviewed was the series of Branch studies generated by the cancer mapping project.

OTHER ACTIVITIES

The Branch continued to provide a liaison for epidemiologic research in the National Cancer Program and for environmental cancer studies being conducted in various agencies in the Federal Government. A great deal of advice and support was given to clinicians, experimentalists, public health officials, and many other groups. Members served on the editorial boards of various journals and on advisory groups and committees connected with cancer centers, several Federal and State agencies, and other national and international activities. Four staff members helped in preparing reports on chemical carcinogens coordinated by the International Agency for Research on Cancer. Several meetings and projects this year were related to bi-national agreements with the People's Republic of China, Italy, France, and Japan. At times the Branch became embroiled in controversial issues and debates (e.g., fluoridation and cancer, low-level radiation, occupational hazards, artificial sweeteners) and on policies and regulations that pose obstacles to epidemiologic research.

Considerable time and effort was devoted, for example, to the field of radiation. Staff members helped to prepare committee reports to assess the risk of mammography for breast cancer (National Council on Radiation Protection and Measurements), to evaluate the biological effects of ionizing radiation (National Academy of Sciences and DHHS), to review the possibility of late effects among U.S. occupation forces stationed in Hiroshima and Nagasaki shortly after the atomic bombings (National Academy of Sciences), to assess the feasibility of studying children living in the southwest during the testing of nuclear weapons during the 1950s and 1960s, and to evaluate the risk of cancer in nuclear workers (Department of Energy).

Special efforts were made this year to identify and utilize epidemiologic resources best available at the national level. Initiatives were taken to stimulate cooperative agreements with several government agencies in charge of routinely collected data resources that can be utilized for epidemiologic studies (e.g., Social Security Administration, Internal Revenue Service, National Center for Health Statistics). Another important activity of the Branch has been the on-the-job training of staff at the post-doctoral level, the supervision of elective periods for medical students, field research opportunities for doctoral candidates at Schools of Public Health, and the assignment of visiting scientists with variable experience in epidemiology. Although the Branch encourages an atmosphere of academic freedom and the development of new ideas and approaches, these are met with critical review and evaluation through several mechanisms: frequent section and branch meetings close contacts with support service and collaborating groups; various formal review mechanisms; several working groups (e.g., data resources, radiation studies, family studies, drug studies); interagency committees; the Clinical Center Review Committee involving clincial investigations; careful scrutiny of questionnaires and their clearance through governmental channels ad hoc external review groups for major studies (e.g., national bladder cancer project, formaldehyde study); the NIH Coordinating Epidemiology Committee; and a variety of advisory bodies that oversee Institute activities, including the Board of Scientific Counselors of the Division of Cancer Cause and Prevention.

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U.S. Cancer Mortality Survey

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P.I.:	T.J. Mason	Head, Population Studies Section	EEB NCI
OTHER:	J.F. Fraumeni, Jr.	Chief, Environmental Epidemiology Branch	EEB NCI
	R.N. Hoover	Head, Environmental Studies Section	EEB NCI
	W.J. Blot	Head, Analytical Studies Section	EEB NCI
	B.L. Stephenson	Computer Specialist	EEB NCI
	R.I. Ramsbottom	Computer Specialist	EEB NCI
	A.E. Blair	Epidemiologist	EEB NCI
	L.W. Pickle	Mathematical Statistician	EEB NCI
	H.M. Hayes, Jr.	Veterinarian	EEB NCI

COOPERATING UNITS (if any)
National Center for Health Statistics, Bureau of the Census, National Oceanic and Atmospheric Administration

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SECTION
Population Studies Section

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SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this study is to examine the cancer mortality experience in the United States relative to cancer etiology. Special emphasis is placed upon the selection of areas in the U.S. for intensive study. Publications from this area of interest have facilitated the design of ongoing analytical investigations to test specific etiologic hypotheses. Rates for multiple myeloma were found to be highest in urban areas and lowest in rural areas; positive associations were seen with indices of socioeconomic level and percentage of residents with Scandinavian ancestry. Leukemia mortality rates were high among whites in the North and South Central States, among nonwhites in the Northeast and Midwest. These geographic patterns persisted after adjusting for urbanization and socioeconomic gradients and for ethnic correlations. Occupational determinants may be involved. A significant positive correlation was seen between PMRs for canine bladder cancer and the overall level of industrial activity in counties with veterinary medical teaching hospitals. Canine bladder cancer could be a sentinel condition whose investigation in certain locales might lead to the early identification of carcinogenic hazards in the general environment.

PHS-6040
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Project Description

Objectives: To examine the cancer mortality experience in the United States relative to cancer etiology. Special emphasis is placed upon the selection of areas in the U.S. for intensive study.

Methods Employed: This project involves computer analysis of over 6 million death certificates by site, sex, race, state, and age. The investigation is ongoing, updated each year, and expanding. Data for all causes of death are utilized from 1968.

Major Findings: A total of 68,400 whites and 10,533 nonwhites were reported to have died from multiple myeloma (MM) in the continental United States between 1950 and 1975 (excluding 1972 because of incomplete case ascertainment). Age-adjusted mortality rates for nonwhites were approximately twice as high as for whites. During the 25-year period of this survey, there was a two- to three-fold increase in MM mortality. The increase was seen in both races, but was greater in nonwhites than whites and primarily occurred in people over 55 years of age. The increases were uniform in all geographic regions and urban/rural categories. MM mortality from 1950-1969 was correlated with geographic, demographic, and occupational factors at the county level. The rates were highest in the Far West and Mid-Central regions for whites and in the Northeast for nonwhites. Urban areas had the highest rates and rural areas had the lowest, and positive associations were seen with indices of socioeconomic level and the percentage of residents with Scandinavian ancestry. For white males, MM mortality rates were elevated in areas with high petroleum and paper production, and a slight increase was seen in furniture manufacturing areas.

Age-adjusted mortality rates for leukemia during 1950-1969 were correlated by race and sex with demographic, industrial, and agricultural data for 3056 U.S. counties. Despite relatively uniform mortality across the country, there were high rates among whites in the North and South Central States, and among nonwhites in the Northeast and Midwest, with correspondingly lower rates in the Southeast. The regional excesses were limited to people over 55 years of age. When cell types of leukemia for 1968-1971 were analyzed, some clustering of acute myeloid leukemia was seen in the central part of the country. The geographic patterns persisted after adjusting for urbanization and socioeconomic gradients, and for ethnic correlations, particularly involving residents of Scandinavian, German, and Russian descent. Occupational determinants may be partly involved in view of geographic correlations with certain food industries, and other industrial and agricultural indicators.

Proportional morbidity ratios (PMR) were calculated for cancers, by site or type, in 8,760 pet dogs seen at 13 veterinary medical teaching hospitals in the U.S. and Canada. A significant positive correlation was seen between the PMRs for canine bladder cancer and the overall level of industrial activity in the host county of the hospital. An analysis of mortality from bladder cancer among white men and women in the same U.S. counties showed similar correlations with industrial activity. Canine bladder cancer could be a sentinel condition whose investigation in certain locales might lead to early identification of carcinogenic hazards in the general environment.

For some time, it has been known that the risk of various cancers may differ strikingly from one continent to another. Indeed, these variations have served as the basis for a rough estimation of the burden of environmentally induced cancer in our society. Compared with international patterns, differences in cancer risk within any country seem much less pronounced. Nevertheless, geographic variations in the United States, such as those revealed by the National Cancer Institute's county-by-county survey, and ethnic and religious differences, such as those highlighted in a recent workshop on populations at low risk of cancer, are providing useful clues to cause of cancer.

The geographic patterns of cancer in the U.S. are varied and provocative, providing a series of etiologic clues, many of which are now being pursued. As in China, special interest has focused on the unanticipated clusters of counties with elevated mortality for particular cancers. These clusters may be considered as "smoke signals" to environmental hazards that await identification through appropriate epidemiologic and mutlidisciplinary studies. The geographic studies undertaken by the U.S. and China provide substantial opportunities for bi-national cooperative endeavors. In all likelihood further discoveries will be hastened by closer communication, and by collaborative and parallel efforts designed to unravel the risk factors responsible for the geographic pecularities of cancer in both countries.

Significance to Biomedical Research and the Program of the Institute: This survey provides a continually expanding data set which has generated specific etiologic hypothesis concerning cancer. The capability of subdividing the data set into specific racial and geographic subsets, e.g., county level analyses, provides an opportunity to also test specific etiologic hypotheses.

Proposed Course: The project will continue to pursue etiologic questions and specifically will address the dynamic changes of rates for malignancy as a function of calendar time and geography.

Publications:

Blattner, W.A., Blair, A., and Mason, T.J.: Multiple myeloma in the United States, 1950-1975. Cancer (In press)

Blair, A., Fraumeni, J.F., Jr., and Mason, T.J.: Geographic patterns of leukemia in the United States. J. Chron. Dis. 33: 251-260, 1979.

Hayes, H.M., Jr., Hoover, R., and Tarone, R.E.: Bladder cancer in pet dogs: A sentinel for environmental cancer. Am. J. Epidemiol. (In press)

Fraumeni, J.F., Jr.: High-rate cancers among low-risk populations. J. Natl. Cancer Inst. 65: 1187-1189, 1980.

Blot, W.J. and Fraumeni, J.F., Jr.: Geographic patterns of cancer in the United States. In Marks, P. (Ed.): Cancer Research in the People's Republic of China and the United States of America. New York, Grune & Stratton, 1980, pp. 65-77.

CONTRACT IN SUPPORT OF THIS PROJECT:

ORI, INC. (NO1-CP-01054)

Title: Biomedical Computing: Design and Implementation

Current Funding Level: $708,645

Man Years: 21

Objective: To provide systems design analysis and programming for intramural research projects.

Methods Employed: Research projects are reviewed for priority, and appropriate managers, analysts, and programmers are assigned tasks. A team of assistant project officers monitor the performance of the contractor and are instrumental in projecting future needs.

Major Contributions: Almost all recent publications from the Branch have had tasks performed by the contractor to facilitate their completion. The team approach that has evolved appears responsible to the varied needs of the Branch.

Proposed Course: To provide comparable support through September, 1983.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04401-05 EEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Immunologic Factors in Cancer Etiology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R.N. Hoover	Head, Environmental Studies Section	EEB NCI
Other:	J.F. Fraumeni, Jr.	Chief	EEB NCI
	T.J. Mason	Health Statistician	EEB NCI
	M.H. Greene	Clinical Investigator	EEB NCI

COOPERATING UNITS (if any)

University of Minnesota; Immunodeficiency Cancer Registry: Veterans Follow-up Agency of the National Academy of Sciences

LAB/BRANCH
Environmental Epidemiology Branch

SECTION
Environmental Studies Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to identify and study populations with altered immunologic states experiencing unusual rates of malignancy. Risks of cancer of different sites are quantified for various groups of such patients, compared with each other, and characteristics and possible determinants of unusual risks are sought. The populations studied include renal transplant recipients, patients with inherited and acquired immune deficiency syndromes, and groups of immunostimulated persons. Both cohort studies of these groups of patients and case-control studies of such patients who also developed cancer are being conducted.

PHS-6040
(Rev. 2-81)

Project Description

Objectives: (1) To identify and study popultions with altered immunologic states experiencing unusual rates of malignancy. (2) To study these populations in order to identify the characteristics and possible determinants of any unusual risk.

Studies and Methods Employed: (1) Further analyses concerning 16,000 patients who received renal transplants. The numbers of cancers observed among various subgroups of this population were compared to that expected based on cancer incidence rates prevailing in the general population. These analyses included a separate evaluation of the risk of nonmelanotic skin cancer in this population and a search for unusual space-time clustering of lymphomas.

(2) A collaborative arrangement has been established with the End-Stage Renal Disease Program of Health Care Financing Administration to evaluate cancer risks among transplant recipients and patients on dialysis. The study population currently includes over 50,000 dialysis patients and more than 5,000 transplant patients. Survival analyses have been carried out, and detailed analyses of the cancer experiences of this population are currenly underway.

(3) The collaboration with the University of Minnesota continues via the contract mechanism (NO1-CP-43384). In the past year, a number of separate analyses have been done in an attempt to characterize the risk of cancer among patients with genetically determined immunodeficiency diseases.

(4) The follow-up study of the malignancy, mortality, and reproductive experiences of 3,000 former employees of Ft. Detrick in Frederick, Maryland, has continued. A large proportion of these workers fall into a group which would be considered "hyperimmunized." Extensive attempts to track down the vital status and the current address for those living have been performed for those individuals who were not readily contacted based on the information received from Ft. Detrick. The data are currently being coded for computer analysis.

(5) A study has been conducted of the clinlical and histological characteristics of malignant melanoma arising in transplant recipients.

(6) A follow-up study of patients with Hansen's disease, previously conducted by the Branch, has been updated. Approximately 1,700 patients with Hansen's disease hospitalized at one institution from 1930 onward have had their clinical records abstracted and have been followed up for mortality and for cancer incidence. These data are currently under analysis.

(7) Work has continued on the creation of longitudinal medical histories for all persons admitted to Veterans Administration Hospitals since mid 1963. This project is in the phase of systems design and verification of the completeness of information. This resource will provide an ever-expanding data base to be used for analytical studies on persons whose exposure and/or outcome diagnoses, as well as procedures, are of specific interest.

Major Findings: (1) There is a 25-fold excess risk of lymphoma among renal transplant recipients. This excess appears within a year of transplant and the tumors show an unusually high frequency of brain involvement. This risk is lower among those who have been among all recipients over time. Other tumors occur at approximately twice the expected frequency. This excess appears more gradually following transplantation and is due to excesses of cancers of the bladder, lung, liver and bile ducts, soft tissues, and malignant melanomas.

(2) Transplant recipients also have an excess risk of nonmelanotic skin cancer. This excess is restricted to squamous cell lesions, and there is no excess of basal cell cancer. The excess increases with increasing interval from transplant and occurs in both high- and low-risk areas for skin cancer. No association with any particular underlying renal disorder was evident, nor was there any significant relationship with any characteristic of donor or recipient on which information was available.

(3) Persons with genetically determined immunodeficiency syndromes have a high risk of lymphomas, similar to that among transplant recipients. These patients also appear to be at high risk of Hodgkin's disease and lymphatic leukemia in children. In addition, they also have elevated risks of soft tissue sarcomas, malignant melanomas, and stomach cancer (adults). Among children with non-Hodgkin's lymphoma in this population, surface marker characteristics indicate that these are primarily B-like phenotype, in distinction to the predominanately "null" or T-phenotype of unselected children with non-Hodgkin's lymphoma. In an extensive study of Wiskott-Aldrich syndrome by the meticulous documentation of tumor occurrence in affected kindreds, the lifetime attack rate for cancer in affected patients appears to be approximately 15% (the median survival of children with this condition for the time period studied is around 6 years).

Significance to Biomedical Research and the Program of the Institute: Host factors are clearly a major determinant of the response of humans to environmental carcinogenic exposures. One of the components of these factors is the immune status of the individuals involved. Much laboratory research has indicated a central role for the immune system in determining who develops a malignancy among those exposed to a carcinogenic agent. The presence of a number of identifiable groups with markedly altered immune states allows an assessment of these factors in the production of human malignancy. In addition, such human observations have already uncovered major associations which have altered some preconceived notions of the relationship between the immune system and cancer.

Proposed Course: (1) The registry of patients with end-stage renal disease will be utilized to continue and expand the studies of patients receiving immunosuppressive drugs for these conditions, including an attempt to conduct a case-control study of the differences in tissue-typing information for those kidney transplant recipients who have gone on to develop malignancy versus those in whom a malignancy has not developed. This resource will also allow the assessment of the risk of malignancy among patients with chronic uremia, an immunosuppressive condition itself.

(2) The collaboration with the immunodeficiency and cancer registry will continue with periodic evaluation of the data collected. A case-control study will be continued in the next year to assess the severity of immunosuppression and immunostimulation in children with these genetic states and cancer, compared to those with similar genetic conditions who did not develop a malignancy.

(3) Patient populations seen at the NIH with other conditions that result in altered immunity (e.g., SLE) will be identified and followed-up in order to obtain mortality and cancer morbidity information.

(4) The study of hyperimmunized employees from Ft. Detrick, in Frederick, Maryland, will be analyzed.

(5) The anlaysis of the cohort with Hansen's disease will be completed.

(6) Large groups of patients with a variety of diseases involving altered immunologic states will be identified in the VA hospitalization file and followed for subsequent morbidity and mortality due to cancer.

(7) Attempts will be made to identify poppulation groups, other than those receiving renal transplants, who received substantial amounts of immunosuppressive therapy. If appropriate, attempts will be made to establish cancer morbidity and mortality studies in these groups.

Publications:

Greene, M.H., Young, T.I., Clark, W.H.: Malignant melanoma in renal transplant recipients. Lancet 1: 1196-1199, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04410-05 EEB

PERIOD COVERED
October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Studies of Cancer-Prone Families

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W.A. Blattner	Head, Family Studies Section	EEB NCI
	M.H. Greene	Senior Clinical Investigator	EEB NCI
OTHER:	Margaret A. Tucker	Clinical Investigator	EEB NCI
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	James J. Goedert	Research Associate	EEB NCI
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	Donald Henson	Pathologist	BCPC NCI
	Arlene Kantor	Epidemiologist	EEB NCI

COOPERATING UNITS (if any) Medicine Branch, NCI; Surgery Branch, NCI; Clinical Epidemiology Branch, NCI; ORI, Inc.; Tissue Bank, Naval Medical Center; Department of Surgery, Uniformed Services University of the Health Sciences; Meloy Laboratories, Inc.; Westat, Inc.

LAB/BRANCH
Environmental Epidemiology Branch

SECTION
Family Studies Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
9.5	7.5	2.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to conduct and coordinate interdisciplinary studies on cancer-prone families in order to clarify the role of genetic mechanisms and host-environmental interactions in carcinogenesis. Clinical studies of high-risk families have suggested etiologic relationships between cancers, constellations of which may cluster in individuals (multiple primary neoplasms) or in kindreds (familial neoplastic syndromes). Bedside observations and epidemiologic studies have clarified the nature of a premalignant cutaneous mole pattern with important implications for melanoma etiology and prevention, and an association of polymastia with kidney cancer. Laboratory abnormalities have provided insights into the mechanisms of susceptibility to cancer. Examples are the finding of subclinical immune dysfunction in family members predisposed to lymphoma, melanoma, and some gastrointestinal malignancies; immunogenetic markers in acute and chronic leukemia, Hodgkin's disease, and Waldenström's macroglobulinemia; DNA repair defects in familial melanoma and in families prone to a diversity of rare cancers; metabolic defects in familial bladder cancer; cytogenetic abnormalities in kidney cancer families; and in vitro cell kinetic abnormalities in colon cancer-prone individuals.

Project Description

Objectives: To document the occurrence of cancer in high-risk families, and to study such families by clinical, epidemiologic, and laboratory investigations, in an effort to elucidate genetic mechanisms and host-environmental interactions contributing to carcinogenesis. To coordinate the distribution of tissue and blood specimens from such families to interested investigators for etiologic studies by cytogenetic, immunologic, viral, endocrine, biochemical, tissue culture, and other methods. To apply innovative analytic approaches to these studies, including statistical genetic approaches.

Methods Employed: Protocols for study of individual families or groups of families are developed, outlining study aims and methods, and are reviewed by section professionals to maximize efficient use of personnel and laboratory resources. Patients are interviewed with respect to prior medical, occupational, and environmental history, and familial occurrence of cancer and other disorders, and are examined for clinical features associated with heightened risk. Family medical history is systematically documented utilizing a family medical history questionnaire developed by section professionals. Clinical history is documented using vital records, hospital and physician charts, and operative specimens are sought for systematic review by collaborating pathologists. Data are abstracted and systematically entered and verified on a computerized record-keeping system. Specialized questionnaires are developed for documenting specific etiologic information. Biologic specimens are collected from informative members of high-risk families, stored in biospecimen repositories, and transmitted to collaborating laboratories. Analysis includes application of computerized genetic, as well as traditional, approaches.

Major Findings: During the last year, approximately 900 new families were ascertained, bringing the total to over 1,900. This reflects the continued ascertainment of new families with familial melanoma, Hodgkin's and non-Hodgkin's lymphomas, multiple myeloma, renal cell, breast, and ovary cancers, neurofibromatosis, and diverse forms of malignancy. In addition, formal arrangements with the NCI Office of Cancer Communications and with the Deputy Clinical Director of NCI have continued to help channel outside inquiries to the Family Studies Section.

During the last year, the integrated manual and computerized record-keeping system was substantially overhauled to increase the efficiency of data collection and to incorporate precise routines for data verification. A manuscript describing this system was prepared for submission. Investigators inside and outside of the NIH have adopted our methodology for family data organization in their newly-established family studies programs. During the last year, a complete inventory of all families logged into the Family Studies Files was made and major diagnostic features abstracted for computer data entry. This comprehensive diagnosis file allows investigators to search for families with shared or interesting features for more in-depth analysis. Currently, there are computerized records on 21,731 members of 622 families, and data entry is proceeding on families selected for in-depth study. The computerized clinical data base is linked to specimen inventory and laboratory results files, simplifying record keeping and improving opportunities for computer-based analysis.

A major focus of the Family Studies Section has been the study of hereditary cutaneous melanoma (HCM) and the dysplastic nevus syndrome (DNS), now entering its sixth year. Over 400 members of 14 melanoma-prone families have been systematically evaluated. An additional 70 melanoma-prone families have been tentatively identified for future study. Major progress during the past year includes: (a) a more precise characterization of the clinical features of the DNS; (b) the first documentation that hereditary melanoma segregates as an autosomal dominant trait, with reduced penetrance; (c) the first description of in vitro ultraviolet radiation sensitivity in cultured skin fibroblasts of patients with HCM and DNS; (d) the establishment, for the first time, of cell lines in tissue culture derived from dysplastic melanocytes; and (e) the completion of three educational videotapes (for high-risk family members, clinicians, and pathologists) designed to disseminate information on the recognition and management of patients at increased risk of melanoma. Educational materials were sent to all members of study families and their physicians outlining a comprehensive program aimed at early detection and prevention of malignant melanoma. A report of this activity is the subject of a presentation at the Oncology Nursing Society's Annual Meeting. The diagnosis of 31 new primary melanomas in study participants (all of whom had the DNS) both confirmed the association between DNS and HCM and documented the value of this surveillance approach in diagnosing HCM when it is surgically curable. Psychosocial determinants of patient compliance with this surveillance program are being systematically investigated by a Family Studies research nurse as part of her master's thesis. Additional studies demonstrated that dysplastic nevi play an etiologic role in both sporadic melanoma and in those melanomas that develop in renal transplant recipients. Manuscripts currently in preparation include: (a) a clinical summary of the 14 high-risk families; (b) a color atlas detailing the DNS; (c) a report of the UV radiation sensitivity found in these families; (d) a summary of segregation and linkage analyses in the 14 families; (e) a description of dysplastic nevi on the scalp of children from high-risk families; (f) a report describing three patients in whom incomplete removal of dysplastic nevi was followed by the development of invasive melanoma at the biopsy site; (g) the first report on the ultrastructure of dysplastic nevi; (h) descriptions of kindreds prone to melanoma and breast cancer, melanoma and lymphoma, and both cutaneous and intraocular melanoma; and (i) an analysis of melanoma mortality data from the U. S. cancer by county resource. Additional studies underway include evaluation of both humoral and cellular immune function and a dermatoglyphic analysis of high-risk families, and attempts to propagate cultured dysplastic melanocytes in nude mice. A cohort of 400 consecutive patients with melanoma from a collaborating treatment institution and followed for 10 years is being restudied in depth to evaluate the contribution of DNS to their risk and that of family members.

A new syndrome of heritable retinoblastoma, cutaneous melanoma, and dysplastic nevi has been described. Other associated tumors are osteosarcomas, and possibly soft-tissue sarcomas. This assocaiton is informative about the mechanisms of carcinogenesis since UV-induced tumors, as well as ionizing radiation-induced tumors, occur in genetically-susceptible hosts. Clinical and laboratory evaluation of these families is planned.

The syndrome of diverse familial neoplasms, including bony and soft-tissue sarcomas, breast and brain cancers, leukemia, and other tumors, first described by Branch personnel in 1969, continues to be a major research focus. The survey

of 20 such families is nearing completion; and to date, 183 neoplasms have been pathologically documented with a remarkable preponderance of rare neoplasms. Analyses to characterize the demographic features of cases, the genetic mode of inheritance, the histopathologic peculiarities and the survival experience of familial cases will begin following final validation of data. Follow-up of the original four families reveals a strikingly high incidence of rare tumors characteristic of the syndrome in close relatives of the original probands. Subsequent cancers in a black family with features of this syndrome, Turcot's, and familial polyposis suggest common etiologic factors or the overlap of several well-characterized genetic syndromes. Long-term follow-up of one large kindred documented the occurrence of new neoplasms in three family members, including a second primary osteosarcoma which developed in the field of prior radiotherapy for bilateral malignant neurilemmoma. Gamma DNA repair studies performed on skin fibroblasts from this patient and seven other close relatives over three generations showed that cells from clinically affected and high-risk individuals, but not spouses, are resistant to cell killing. Biochemical characterization of this novel phenotype has important implications for understanding mechanisms of carcinogenesis. Studies on cell strains from more distant branches of this family and from probands of five other well-characterized families should help to define the utility of this phenotype as a possible marker of cancer risk and may correlate with the clinical impression of increased susceptibility to environmental carcinogens, including ionizing radiation in this syndrome.

Immunologic parameters have been evaluated in families susceptible to a variety of lymphoreticular neoplasms.

In follow-up of previous work in which an association between familial Hodgkin's disease and HLA-Bw35 was reported, six kindreds in which two or more Hodgkin's disease patients had survived have undergone extensive evaluation of the major histocompatibility complex (MHC). Preliminary analysis revealed that two Dr typing sera appeared to identify a genetic marker in these families which segregated independently from known HLA-A, -B, -C, and -Dr specificities. More recent data confirms this observation and defines this new specificity as the MT-1 phenotype. MT is a recently-described MHC locus which maps in the Dr region of the MHC genome. This observation represents the first disease association with the MT locus and explains the results of our earlier report, since Bw35 and MT-1 are in linkage disequilibrium. Studies are now in progress to evaluate <u>in vitro</u> suppressor cell function in members of these families.

An unusual family with Waldenström's macroglobulinemia in a father and three of his four children, and autoimmune disease in numerous other family members, appears to represent an instance where a defective immune response gene, linked to the major histocompatibility locus (Lod-score 4.86 strongly supports linkage), predisposes to lymphoproliferation and autoimmunity. Further studies of regulatory cell function are under way to evaluate this association.

Another family under study since 1972 was recently recontacted because of the development of non-Hodgkin's lymphoma in the sixth of nine siblings. A comprehensive protocol aimed at studying immunologic and immunogenetic parameters has confirmed a remarkably high incidence of subclinical immunoglobulin abnormalities in numerous branches of this large family, with a tendency to cluster in certain sibships. A suspected defect in immunoregulatory function will be evaluated

utilizing monoclonal reagents that characterize functional subsets on a fluorescence-activated cell sorter (FACS-II) and by in vitro study of immunoglobulin synthesis.

Families prone to chronic lymphocytic leukemia are systematically being studied to establish whether cell surface markers are shared between familial cases. In one family, several members with normal white blood counts and lymphocytosis are being studied with the FACS-II to determine if early perturbation in B-cell clones can be detected as a precursor to chronic lymphocytic leukemia (CLL). Follow-up of a family in which a father and four of his five offspring were diagnosed with CLL confirms that certain cell surface features are shared among the cases. Surprisingly, one of the cases with classical CLL in 1974 has undergone a spontaneous remission, confirmed by FACS-II analysis. Immunogenetic study of this family reveals the presence of shared B-cell typing reactions which segregate independently from known HLA-A, -B, -C, and -Dr specificities in the cases, but not in normal family members. Collaboration with recombinant DNA geneticists at NIH and the University of Wisconsin is underway, with plans for cloning the immunoglobulin genes from familial cases in order to study their detailed structural organization.

The first familial aggregation of hairy cell leukemia (HCL) was discovered, evaluated, and reported. The two HCL cases were HLA-identical brothers from a sibship of eleven adults; none of the unaffected sibs shared both HLA haplotypes found in the two cases. Other hematologic and immunologic studies in the family were normal. The data suggested that there may be an HLA-associated disease susceptibility locus for HCL. To explore this possibility further, a detailed laboratory study of this family is now underway, focusing on a more sophisticated assessment of HLA and on cell surface marker studies employing the FACS-II.

Cells from members of a family prone to acute lymphoblastic leukemia were studied at the recent International Tissue Typing Workshop and by collaborators at Georgetown University. Results of this evaluation demonstrated that the affected sibs were homozygous for Dr4-related reactivities and certain MT specificities. This result validates the original hypothesis that homozygosity for major histocompatibility determinants is associated with risk for leukemia, a conclusion that is emerging in the recent medical literature.

Among 526 patients with cutaneous T-cell lymphomas, 21 reported first-degree relatives with lymphoproliferative or hematopoietic malignancies. Intensive case findings, coupled with pathology review, confirmed 29 such cases in these 21 kindreds. Hodgkin's disease was over-represented in this group, accounting for one-third of the cases. These data form the basis for the hypothesis that genetically-determined immunoregulatory abnormalities may represent a shared pathway of oncogenesis in diverse lymphoproliferative and hematopoietic malignancies. To pursue this and other etiologic hypotheses, a population-based case-control study of cutaneous T-cell lymphoma is now well into the planning stages, with field work scheduled to begin in June 1981.

In collaboration with the Department of Pediatric Hematology of the University of Illinois (Chicago), a family prone to myeloproliferative disease has been evaluated. Two sisters and a niece had this disorder, while the niece's father and a distant cousin were found to have splenomegaly of undetermined etiology. Laboratory studies thus far, including cytogenetics, colony-forming assays, HLA studies

and in vitro immune function, have been unrevealing. The family will be reported clinically while additional analyses of the laboratory data are completed.

In a recent report, a relationship between an inborn cytogenetic abnormality (familial 13:18 translocation) and risk for cancer is suggested by the occurrence of acute leukemia and stomach cancer in carriers of this marker chromosome. Infertility in all males and some females and ovarian cysts and teratomas suggest that this heritable abnormality also affects gonadal function.

A comprehensive review of the epidemiology of multiple myeloma and Waldenström's macroglobulinemia, with special emphasis on familial and genetic aspects, showed that different genetic mechanisms operate in these conditions. For macroglobulinemia, a dominantly inherited predisposition to autoimmunity and lymphoproliferation is suggested; while for myeloma, a recessive factor (possibly associated with HLA) may explain the pattern. Family histories are being collected as part of ongoing case-control studies of macroglobulinemia and myeloma in hopes of helping clarify these questions, and immunogenetic correlates are being evaluated in selected cases.

A review of the epidemiology of non-Hodgkin's lymphoma (NHL) identified two distinctive patterns of familial aggregation for the tumor: (1) teenage male sibs with extra-nodal (primarily gastrointestinal) NHL and (2) adult female sibships with nodal NHL. The many conditions associated with an increased risk of NHL were reviewed, and an etiologic model proposed in which defective immunoregulatory function and chronic antigenic stimulation appear to underlie many of the known lymphoma precursor states. These data were presented at an NIH Combined Clinical Staff Conference on Recent Advances in NHL.

Studies of the relationship of autoimmunity to cancer are continuing with the evaluation of 30 kindreds identified through a proband with dermatitis herpetiformis and 25 kindreds with gluten sensitive enteropathy. In a preliminary report of these studies, family members were found to have an increased incidence of autoimmune disorders in first- and second-degree relatives compared to a cohort of spouse control families. However, the patterns of disease differed in the two study groups, suggesting different immunogenetic determinants. There was no excess of gastrointestinal, lymphoproliferative, or other forms of malignancy compared to the expected incidence for the U. S. population.

The bedside observation that several patients with renal cell cancer had supernumerary nipples lead to a case-control study conducted in collaboration with investigators from the Clinical Epidemiology Branch, NCI. It was found that 19 percent of kidney cancer patients (compared with no control patients and 0.3 case expected from population estimates) had supernumerary nipples. Other minor congenital anomalies which appeared in this group included duplicate renal arteries, renal cysts, and a variety of genitourinary abnormalities. Family histories confirmed an increased likelihood for similar abnormalities, as well as clusters of renal and brain cancers in relatives. A report of these findings is in press. A follow-up case-control study to confirm these findings, to ascertain the diagnostic accuracy of supernumerary nipples, and to better define the host factors involved in cancer of the kidney is in progress. Data from the Hanes dermatologic survey have been used to define a population-based estimate of supernumerary nipples and to calculate that this anomaly carries a relative risk of 50 for renal cancer.

In one family with kidney cancer and nipple anomalies in brothers, a possible deletion of the terminal segment of the long arm of chromosome 8 was detected in both cases, but not in their brother with supernumerary nipples alone. Similar deletions, as well as other constitutional cytogenetic abnormalities, were identified in several members of a cohort of kidney cancer cases with positive family histories, bilateral disease, or exceptionally young age at onset. This cohort was identified through a large case-control study of kidney cancer patients at the University of Minnesota. Confirmatory evaluation of patients and their close relatives is to be undertaken, and a sampling of cases from the survey population will be examined for minor congenital anomalies.

A search was made for cancers among offspring and siblings of 149 Connecticut-born children with Wilms' tumor reported to the Connecticut Tumor Registry during 1935 to 1973. Nasopharyngeal rhabdomyosarcoma developed in the daughter of a man with unilateral Wilms' tumor that also affected his sister. Hodgkin's disease developed in the daughter of a woman who had unilateral Wilms' tumor. One other patient had a sibling with Wilms' tumor and three had a sibling with other cancers (two Hodgkin's disease, one testicular seminoma). The survey suggests an excess risk of other forms of cancer among the progeny and siblings of Wilms' tumor patients.

A cohort of familial testicular cancer cases has been identified through a case-control study conducted by Branch personnel. A comprehensive evaluation of male first- and second-degree relatives reveals a high incidence of clinical and subclinical (utilizing testicular ultrasound) testicular and urogenital abnormalities. A laboratory protocol evaluating genetic, cytogenetic, and hormonal influences is underway. A survey of knowledge and attitudes about testicular cancer is being implemented by the Family Studies nurse epidemiologist to aid in developing effective approaches for primary prevention and early detection through routine self-examination.

Follow-up of a bladder cancer family first reported in 1967 revealed the development of an additional case. In all cases, cigarette smoking was implicated in the development of their cancer; and in one case, heavy saccharin usage might have acted as a tumor promoter. All three cases had a rapid N-acetyl transferase phenotype, and two had detectable urinary N-nitrosodibutylamine. These findings suggest the interaction of host susceptibility with environmental exposures.

Follow-up of a cohort of 16 ovarian cancer-prone families, including 7 previously reported, continues with special emphasis on review of pathology material aimed at clarifying a previously-reported preneoplastic lesion in the ovaries of high-risk family members. A collaborative arrangement for studying the putative linkage between the glutamate-pyruvate transaminase locus and the propensity to breast carcinoma, ovarian-breast carcinoma, and/or ovarian carcinoma is planned. When high-risk individuals are identified, a laboratory protocol to investigate endocrinological status of high-risk family members will be implemented.

Questionnaires filled out by Clinical Center patients with breast carcinoma have been abstracted for various risk factors, including family history; and results of estrogen receptor levels on tumor biopsy specimens are being evaluated to confirm a possible link between familial breast cancer risk and receptor positive status.

A recent hypothesis suggests that alterations in the levels of the pineal gland hormone, melatonin, may be associated with breast cancer risk. A pilot study by the investigators in NCI's Division of Cancer Treatment and the National Institute of Child Health and Human Development suggests some clinically-affected women show an altered circadian pattern for melatonin. The study will be extended to familial breast cancer pending further results of the pilot study.

A young male member of a family prone to diverse malignancies developed breast cancer following thymic irradiation in infancy. In vitro studies of cultured skin fibroblasts from members of the family reveal a significant sensitivity to both ionizing radiation and bleomycin in the proband. The data are consistent with an etiologic role for this radiation sensitivity in the pathogenesis of the proband's breast cancer. A familial cancer predisposition may have contributed further to this patient's disease susceptibility. The case is an excellent example of the role of host-environmental interactions in cancer pathogenesis.

Studies carried out in collaboration with Dr. Martin Lipkin at Memorial Sloan-Kettering Institute on colon cancer-prone families have suggested the utility of actin cable analysis as an in vitro marker of polyposis risk, while colonic washing CEA levels, although frequently abnormal, do not appear to be highly correlated in many cases. In vitro study of colonic cell growth characteristics demonstrates that a shift in proliferative compartment appears to signal a mutational event identifying a premalignant change in both polyposis and non-polyposis colon cancer-prone families. Further studies are planned to evaluate this growth abnormality in high- and low-risk groups from selected populations utilizing more precise statistical approaches suggested by Branch personnel.

As part of an interagency collaboration between NCI/EEB and the Center for Disease Control, a series of special studies of cancer in Alaskan natives is now under way. An important phase of the project is ascertainment and evaluation of kindreds prone to hepatocellular and nasopharyngeal carcinoma. Protocols for the clinical/laboratory evaluation of these families have been developed, and specimens from three kindreds have been obtained for immunogenetic and immune function studies.

Under a recently-terminated contract [Litton Bionetics,Inc. (NO1-CP-61016)], a battery of immunologic tests, including functional and cell population tests, were conducted on 726 members of cancer-prone families. In many cases, repeat testing was performed. In addition, a variety of tumor-specific assays were also performed. Data on these patients have been edited and analysis is underway utilizing the Family Studies computer system. Preliminary analysis reveals that affected cancer patients and their first- and second-degree relatives have decreased numbers of T-cells and depressed response in mixed leukocyte culture. Multivariate analysis to characterize potential confounding variables is planned with emphasis to be placed on studying T-cell populations. Statistical approaches developed to apply nonparametric discriminant analytic approaches to these data were the subject of a publication by the SAS Institute.

A parallel data set of HLA genetic marker data on many of the same family members tested immunologically is currently being edited and verified. This data set will allow for analysis of the association of HLA type to diverse forms of

familial cancer. In addition, the genetic marker data adds another parameter that can be correlated with immune response.

A report describing the metastasis of maternal cancer to the fetus was published. One section member participated in the evaluation of an outbreak of varicella-zoster illness among National Cancer Institute, Medicine Branch patients. A possible carcinogenic role for polyethylene glycol, a potent cell fusogen used in human medications, was suggested in another publication.

Significance to Biomedical Research and the Program of the Institute: Studies of cancer-prone families may help to detect genetic mechanisms and host-environment interactions in carcinogenesis and also help to identify those individuals most likely to benefit from screening programs aimed at early diagnosis of cancer. These unique, high-risk populations offer an opportunity to evaluate patient and physician education techniques and assess the impact on health status of the patients over time.

Proposed Course: The Family Studies Unit was formally made a Section during the last year. The same basic methodologies will be applied, emphasizing a more systematic approach to studying groups of families with shared features, rather than individual families. The development of a computerized data base and the availability of statistical genetic techniques have added impetus to this more targeted approach. Laboratory collaboration will continue to expand, utilizing the fibroblast cell strains, sera, and other biospecimens collected over the last ten years. Immunologic analysis, utilizing the FACS-II, the availability of recombinant DNA approaches for gene cloning, and cytogenetic analysis with prophase banding are areas of laboratory collaboration that show particular promise.

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Pandey, J.P., Tung, E., Mathur, S., Namboodiri, K.K., Wang, A.C., Fudenberg, H.H. Blattner, W.A., Elston, R.C., and Hames, C.: Linkage relationship between variable and constant region allotypic determinants of human immunoglobulin heavy chains. Nature 286: 406-407, 1980.

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Contracts in Support of This Project

MELOY LABORATORIES, INC. (NO1-CP-91000)

Title: Skin Fibroblast Repository for Patients at High Risk for Cancer

Current Funding Level: $67,000

Man Years: 1.5

Objectives: Maintain and develop a repository of skin fibroblast strains on high-risk patients and members of families at high risk for cancer.

Methods Employed: Primary skin biopsy explants are processed using standard tissue culture techniques and screened for contamination. These cell strains are frozen in liquid nitrogen and distributed to collaborating investigators on written request.

Major Contributions: Approximately 350 new specimens were submitted to the repository over the past year. Approximately 200 separate cell strains were sent to collaborating investigators at 14 different institutions. A number of publications resulted from the use of these cell strains, including a series of studies which focused on DNA repair response to ultraviolet and gamma radiation. Abnormalities were identified in a family prone to acute myelogenous leukemia, a patient with radiation-induced male breast cancer, patients with hereditary cutaneous malignant melanoma, and patients from a family prone to a diversity of bony and soft-tissue sarcomas, brain and breast cancers, and leukemia. The availability of specimens on multiple generations of affected families have proven especially valuable for evaluating the suspected genetic mode of transmission of susceptibility in some cases. Cells from one family proved useful in defining the presence of a variant of human non-muscle tropomyosin.

Proposed Course: A new competitive procurement is planned to establish a successor to this contract. Similar support activities with a broader range of collaborating investigators is planned in hopes of making this valuable resource more readily available.

Date Contract Initiated: October 1, 1980

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 CP 04411-05 EEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Cancer and Related Conditions in Domestic Animals: Epidemiologic Comparisons with Man.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Howard M. Hayes, Jr.	Staff Associate	EEB NCI
OTHER:	Joseph F. Fraumeni, Jr.	Chief, EEB	EEB NCI
	Robert Hoover	Chief, ESS	EEB NCI
	Karen L. Milne	Staff Associate	EEB NCI

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LAB/BRANCH
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SECTION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.8	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The continuing purpose of this project is to identify animal models applicable to further research into etiology of cancer in humans. Additionally, as cases accumulate, the probability increases that domestic pets with some types of spontaneous cancers can be identified as representing the effects of low-level environmental exposure to carcinogenic agents. The frequency of cancer in these animals would serve as a warning of general environmental hazard to man. The topics of current interest include: 1) environmentally influenced cancer in dogs relative to the level of industrialization in their county of residence; 2) the epidemiologic features of splenic neoplasms, gastro-intestinal cancer, colorectal cancer, and prostatic cancer in dogs; 3) a case-control study of the long-term effects of Promone and Ovaban in dogs; and 4) morbidity among military working dogs compared to that in pet German Shepherds.

PHS-6040
(Rev. 2-81)

Project Description

Objectives: To investigate the distribution of cancer and related conditions in domestic animals in order to 1) clarify etiologic factors in humans, 2) identify animal models useful in research, and 3) identify sentinels which may act as early predictors of environmental hazards to man.

Methods Employed: Animals with the disease under investigation are identified when possible from the medical abstract records in the Veterinary Medical Data Program. A comparison population-at-risk is constructed from all patients seen by participants during the same time period under consideration. The diseased animals are characterized by relative risk techniques for various factors (i.e., age, breed, sex). Other analytical techniques employed may include case-control comparison for factors associated with the disease in man. Other animals are studied wherever another resource is available (i.e., data base of the Armed Forces Institute of Pathology).

Major Findings: 1) The proportional morbidity of various cancers in the dog was correlated to the amount of industry in the locale of the dog's home (assessed by zip code). Canine bladder cancer had the strongest positive correlation of any cancer with industrial activity. Further analyses showed similar correlations between human mortality from bladder cancer and industrial activity in the same geographic locales, suggesting that the pet dog may be a sentinel for general environmental hazards.

2) A study of 132 cases of mammary tumors in cats indicates mammary carcinoma is the most common histologic cell-type. Spayed cats have 0.6 the relative risk of intact females; ovarian carcinoma, the most common second primary cancer with mammary cancer in the dog, was not seen concomitantly with feline mammary carcinoma. Most data suggest the cat may be an appropriate model for improving our knowledge of human breast cancer.

3) A study of canine hypothyroidism identified nine breeds with excessive risk, showed that risk by age was greatest among young dogs of high-risk breeds, and that spayed female dogs were at higher risk than intact females.

4) Study of systemic mycoses in dogs indicated different but strong seasonal trends for blastomycosis, coccidioidomycosis, and histoplasmosis. Each disease was more prevalent among males.

5) Ongoing analyses have revealed a strong male risk in dogs for colorectal carcinoma, a vulnerability among several breeds which may, in part, be based on being large dogs, and a lack of a direct association with prior adenomas.

6) Investigation of canine biliary carcinoma showed no apparent genetic predisposition although a sex differential was suggested. Some evidence was available suggesting recent infestation with intestinal parasites occur more frequently than expected, thereby possibly playing a role in the etiology.

7) A survey indicated most diaphragmatic defects in the dog are pericardial diaphragmatic hernias. Many of these are associated with cardiac defects.

8) A predisposition to congenital deafness and hearing impairment, to a lesser extent, was identified in the Dalmatian and six other canine breeds, many of which are prone to pigment abnormalities of the coat and eye. Although concomitant eye defects were common, they did not appear to be part of the familial deafness syndrome.

9) The current status of veterinary cancer investigations was reviewed by comparing the epidemiologic features of canine, feline, and human cancers at ten body sites. Considerable similarity was evident between the cancer experience of the dog and man.

10) A review was prepared of the epidemiology and therapeutic approaches to canine mammary neoplasm.

11) A review of surgical techniques for canine ovariohysterectomy was presented, with the therapeutic advantages and disadvantages of the surgical procedure.

12) A review was presented of various domestic animal species that have been used as sentinels of human health hazards.

Significance to Biomedical Research and the Program of the Institute: Canine bladder in pet animals appears to be strongly associated with increasing amounts of industrial activity in U.S. counties where data are available from veterinary teaching hospitals and clinics. We believe spontaneous bladder cancer in the dog generally represents the effects of environmental carcinogens; monitoring the frequency of canine bladder cancer could serve as a sentinel to emerging hazards to comparison humans. Investigation of canine colorectal carcinoma, feline mammary carcinoma, and canine hypothyroidism demonstrated specific sex hormone dependent association; each disease could serve as an appropriate model for further research applicable to humans. Studies of deafness and diaphragmatic hernia in the dog each showed general clinical features similar to that in man, particularly canine deafness. The identification of certain breeds with apparent genetic predisposition offers additional models for furthering our understanding of congenital deafness in people.

Proposed Course: The methods employed and projects listed will be continued next year.

Publications:

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04412-05 EEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Carcinogenic Effects of Therapeutic Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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LAB/BRANCH
Environmental Epidemiology Branch

SECTION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to study the long-term health effects of therapeutic drugs as they may relate to carcinogenicity. Cohort studies of exposed groups are conducted, as well as case-control studies of selected cancer sites which involve lifetime drug use histories. Emphasis in the past year has been on the evaluation of various types of estrogenic preparations, immunosuppressive drugs, and cancer chemotherapeutic agents.

PHS-6040
(Rev. 2-81)

Project Description

Objectives: (1) To study the long-term effects of therapeutic drugs in humans in order to identify drugs affecting risk of malignancy, and the characteristics of these risks. (2) To review what is known about the carcinogenic potential of drugs in order to identify those requiring study in humans.

Studies and Methods Employed: (1) A retrospective cohort study of 1,891 women treated with conjugated equine estrogens for menopause. This is a study of women so treated sometime between 1940 and 1969 and followed up through the end of 1972. This is being done in collaboration with a gynecologist at the University of Louisville. Currently an update of the follow-up is being conducted in order to bring the cohort follow up through 1979.

(2) The data from a case-control study of breast cancer in one pre-paid health plan were analyzed for the relationship between the use of estrogens for the climacteric and the subsequent risk of breast cancer.

(3) A case-control study of breast cancer among mammography screening program participants has included an evaluation of the influence of estrogens and oral contraceptives on breast cancer risk (see Project No. Z01-CP-04501-04). Current analysis is assessing the effect of antihypertensive agents and tranquilizers on risk of breast cancer.

(4) Study of the long-term effects of the immunosupressive drugs described in detail in Project No. Z01-CP-0441-04-EEB, "Immunologic Factors in Cancer Etiology."

(5) A population-based, record-abstract, case-control study of approximately 600 cases of ovarian cancer and 700 controls from two pre-paid health plans is currently under analysis to determine the relationship between the use of a number of therapeutic drugs and the risk of this malignancy. The drugs being evaluated include estrogens, oral contraceptives, major tranquilizers and other drugs affecting the pituitary-ovarian axis.

(6) A population-based, record-abstract, case-control study of cases of breast cancer was continued in women who had undergone a bilateral oophorectomy prior to breast cancer diagnosis, and in control women who also had a bilateral oophorectomy. This is being done in two pre-paid health plans in order to evaluate the risk of breast cancer associated with the use of replacement estrogens by women having had a bilateral oophorectomy.

(7) A "case-control" study, where the "cases" are NCI Medicine Branch breast cancer patients whose tumors have estrogen receptors and the "controls" are those breast cancer patients without receptors, is currently being analyzed. Breast cancer risk indicators and history of exposure to estrogens and oral contraceptives are being related to the absolute level of estrogen receptors of the tumors.

(8) A study of patients with Hansen's disease is currently under analysis. The predominant mode of therapy of these individuals for a number of years has been the drug dapsone. This drug has recently been implicated as a carcinogen in the bioassay program of the NCI. Patients with Hansen's disease are also of interest with respect to their risk of malignancy because of their altered immunologic state (see Project No. Z01 CP 04401-04 EEB).

(9) A case-control study in 4 U.S. cancer registries and in Denmark is ongoing. Approximately 400 persons who developed endometrial cancer as a second cancer following breast cancer therapy are being evaluated along with matched controls. Detailed information is being collected on medical histories and estrogen exposures. The risk of endometrial cancer will be quantified in light of cumulative estrogen use.

(10) A mortality study of a population of 338 women treated with isoniazid for tuberculosis and followed up for up to 23 years (12 years mean) was analyzed.

(11) A systematic evaluation of adjuvant drug therapy for cancer treatment has continued. To evaluate the potential carcinogenic effects of various modalities in the treatment of cancer, information from several NCI-supported cancer treatment protocols is being combined and analyzed. This study is being done in collaboration with the Division of Cancer Therapy. From a survey of NCI-funded protocols, a number of cancer treatment trials were selected for evaluation. Protocol chairmen and statisticians were contacted, available data evaluated, and abstract forms designed to obtain information on second cancers not readily available from computerized data. Contact has been made with the following surgical adjuvant groups: The National Surgical Adjuvant Breast Project, the Gynecologic Oncology Group, the Veterans Administration Surgical Oncology Group, the Eastern Cooperative Oncology Group, the Gastrointestinal Tumor Studies Group and the Southwest Oncology Group. Drugs being evaluated include: thioTEPA, L-PAM, MeCCNU, Cytoxan, and others.

(12) A follow-up study of mortality and cancer incidence in 900 patients treated with alkylating agents for rheumatoid arthritis is currently being analyzed. This is being done in collaboration with investigators at the Cleveland Clinic.

(13) A follow-up study was done on 517 patients with non-Hodgkin's lymphoma treated at the National Cancer Institute to evaluate the risk of second cancers.

(14) The effect of thyroid hormone replacement following treatment for hyperthyroidism has been evaluated in a follow-up study of 3,146 patients and is currently being expanded to include over 20,000 patients (see project Z01 CP 04481-05 EEB).

(15) A follow-up of patients with early stage ovarian cancer participating in three prospective randomized trials (GOG, Princess Margaret Hospital, and the MD Anderson Hospital) was conducted and analyzed for the frequency of second tumors. In an effort to obtain larger numbers of cases so that questions arising from this analysis (e.g., dose-response, radiation/chemotherapy interaction, etc.) can be investigated, data from an additional 6,000 women with advanced stage ovarian cancer are now being analyzed. These data will be added to those already collected. The final study group will include approximately 7,500 ovarian cancer patients.

(16) A case-control study of 203 children with second malignant neoplasms, evaluating the relationship between the therapy they received for their first malignant neoplasm and the development of their second, is currently under analysis.

(17) A study of mortality and the frequency of second cancers related to drug and radiation treatments for Hodgkin's disease is underway using Connecticut Tumor Registry data.

(18) A study of benign breast disease conducted in conjunction with epidemiologists at Oxford University allowed evaluation of the relationship of oral contraceptives to risk (see project no. Z01-CP-04501-04-EEB).

(19) A comprehensive review was performed of the human evidence relating to the risk of neoplasia associated with the use of a wide variety of therapeutic drugs.

Major Findings: (1) Among 345 women with breast cancer and 611 controls, all of whom were members of one pre-paid health plan, the relative risk (RR) associated with ever having used conjugated estrogens was 1.4 ($p=0.03$). There was evidence of a dose-response relationship with three different measures of dose, rising to two-fold for those with long-term use.

(2) Estrogen use was evaluated among 881 menopausal breast cancer patients and 863 controls identified through the Breast Cancer Detection Demonstration Project. Use of estrogens was associated with a relative risk of 1.24, with higher risks observed among users of high-dose preparations. Hormone effects predominated among women who received them following bilateral oophorectomy (RR=1.54), obliterating the protective effect normally associated with the operation. High risks were also observed among oophorectomized women who used hormones in the presence of other risk factors, including nulliparity, family history of breast cancer, and benign breast disease.

(3) Use of immunosuppressive drugs by kidney transplant recipients is associated with a 25-fold excess risk of lymphoma, and lesser excess risks of lung cancer, lower urinary tract cancers, soft tissue sarcomas, cancers of the liver and bile ducts, and malignant melanoma. (See Project No. Z01-CP-04401-04-EEB for a more complete description.)

(4) Preliminary evaluation of the risk of leukemia following alkylating agent use in ovarian cancer patients participating in randomized trials indicates a high risk that apparently follows a dose-response relationship.

(5) Among 338 women treated with isoniazid for pulmonary tuberculosis, no excess cancer deaths occurred (8 observed vs. 8.3 expected) after 23 years (12.9 mean) of follow up. There was an excess of cancer deaths (54 vs. 35.7) among 1,090 patients who did not receive INH, partly due to radiogenic breast cancer resulting from multiple chest fluoroscopies to monitor pneumothorax. Increased deaths from liver cirrhosis (5 vs. 0.8) were observed following INH use, suggesting that chronic as well as acute liver disease may complicate this treatment.

(6) Preliminary analysis of the follow-up of 517 patients treated for non-Hodgkin's lymphoma at the NCI suggests excesses of acute non-lymphocytic leukemia and lung cancer. The leukemia cases seemed to be related to therapy, particularly total body irradiation, whereas the lung cancer excess was not related to a specific treatment. Of special interest is the fact that the leukemia excess was confined to patients with nodular lymphoma and lymphocytic lymphoma.

(7) There was no increased risk of breast cancer among 1,179 patients treated with thyroid hormone for iatrogenic hypothyroidism compared to 1,667 patients not so treated.

(8) An evaluation of 827 patients with Hodgkin's disease revealed no excess risk of heart disease deaths, and no relationship among those who did die of heart disease to prior treatment with the vinca alkaloids (vincristine and vinblatine). This analysis was done in response to a number of case reports inplying a relationship between the drugs and mycoardial infarction.

9) An analysis of 1,227 cases and 1,213 controls participating in the BCDDP revealed no association between a variety of aspects of valium (diazepam) use and the risk of breast cancer, thus providing no support for concerns from laboratory animal experiments about the tumor growth-promoting action of this drug.

(10) The importance of various risk factors for benign breast disorders has been assessed in an analysis of data obtained from a multicenter cohort study of contraceptive use among women in the United Kingdom (the Oxford Family Planning Association Contraceptive Study). The cases comprised all women diagnosed as having any type of benign breast lesion; 74 had fibroadenoma, 211 had histologically confirmed chronic systic disease, 331 had breast lumps not subjected to biopsy and 70 had other disease. Each case was individually matched with another study participant who was free from recognized breast disease. Matching factors were center of recruitment, date of recruitment, age at entry, and continuation in the study. An inverse association was found between use of oral contraceptives and the risk of the first three conditions. Current users of the pill had the lowest risk, particularly when the use was for an extended period. In contrast, past users demonstrated

no reduction in risk. The reduction in risk for chronic cystic disease appeared to relate to the amount of progestogen contained in the pill. No significant association was observed between the risk of any of the conditions and either parity or age at first livebirth. Women of low social class and obese women were at low risk, perhaps reflecting diagnostic biases.

Significance to Biomedical Research and the Program of the Institute: Drug exposure has been one of the most fruitful areas for identification of carcinogens in man and subsequent opportunities for preventive programs and insights into the biologic mechanisms in cancer etiology. In addition, the studies of the long-term carcinogenic effects of anti-tumor drugs is an important part of the evaluation of the efficacy of treatment of various malignancies with these agents.

Proposed Course: (1) The retrospective cohort study of menopausal estrogen users will be continued and updated, and further analyses done on cancer incidence and mortality.

(2) The analyses of the breast cancer case-control studies from the pre-paid health plans will be continued. Additionally, they will include the evaluation of stage and survival information as well as the risks associated with the use of drugs other than conjugated estrogens.

(3) The study of ovarian cancers in pre-paid health plans will be analyzed, and potential for other drug evaluations in these pre-paid plans will be assessed.

(4) Another evaluation of the relationship between a number of drugs and the risk of breast cancer and benign breast disease will be analyzed using the data from the case-control interview study done in conjunction with the Breast Cancer Detection and Demonstration Projects (see Project No. Z01-CP-04501-02-EEB for a more complete description), and through the study of breast cancers in oophorectomized women in pre-paid health plans.

(5) It is intended to continue the systematic monitoring of long-term toxic effects (including carcinogenicity) of a number of therapeutic agents used in the treatment of specific cancers. This will be done in collaboration with the Division of Cancer Treatment, as outlined in the methods. These effects will be supplemented, where appropriate, by observational case-control studies of specifically suspect constellations of double-primary malignancies. These evaluations will involve intensive record abstraction for therapy administered to patients who developed certain combinations of primary malignancies, compared to those with the same first primary who did not develop a subsequent malignancy.

(6) Analyses will be done of the risk of cancer among patients with rheumatoid arthritis, who were treated with alkylating agents. In addition, other non-cancer patient groups who have received substantial amounts of immunosuppressive or cancer chemotherapeutic drugs will be sought, and their cancer risks evaluated, when possible.

(7) The analyses of the mentioned ovarian cancer trials and the extended patient series will be completed, and the data from the NSABP will be obtained and evaluated. From this, and the prior studies noted, a decision will be made concerning a summary paper on the risk of leukemia following various dose levels of alkylating agents.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04480-05 EEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Studies of Occupational Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. Blair	Epidemiologist	EEB	NCI
OTHER:	W. Blattner	Clinical Investigator	EEB	NCI
	K. Cantor	Guest-Worker-EPA	EEB	NCI
	D. Grauman	Computer Systems Analyst	EEB	NCI
	S. Hoar	Epidemiologist	EEB	NCI
	J. Lubin	Health Statistician	EEB	NCI
	B. Miller	Epidemiologist	EEB	NCI
	R. Spirtas	Health Statistician	EEB	NCI
	T. Thomas	Epidemiologist	EEB	NCI
	J. Walrath	Epidemiologist	EEB	NCI
	D. Winn	Epidemiologist	EEB	NCI

COOPERATING UNITS (if any)
Social Security Administration, National Institute of Occupational Safety and Health, U.S. Coast Guard, U.S. Department of Agriculture, Office of Personnel Management, U.S. Air Force.

LAB/BRANCH
Environmental Epidemiology Branch

SECTION
Occupational Studies Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
10.3	6.7	3.6

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Cancer patterns among occupational groups that may reflect exposures in the work environment are identified. Study groups are selected because of 1) exposure to established or suspect carcinogens, 2) reports suggesting an unusual cancer experience, and 3) requests by outside institutions. Resources used include employment and death records maintained by companies, labor unions, professional organizations, and government agencies. Investigations completed during the past year include: 1) proportionate mortality studies of petroleum refiners, embalmers, and professional artists, 2) death certificate case-control studies of leukemia and cancers of the brain, bladder, and lung, and 3) cohort mortality studies of industrial chemists. Other investigations underway, but not completed, include cohort mortality studies of workers having exposure to metal fumes, perchloroethylene, pesticides, wood dusts and paints, formaldehyde, taconite, benzene, and laboratory chemicals. Proportionate mortality studies are being conducted among tobacco manufacturers, potters, leather workers, fur dyers, veterinarians, foresters, plumbers, photographers, and millers. Case-control studies are underway to evaluate the role of farm exposures in the origin of leukemia, lymphoma, soft-tissue sarcomas, and multiple myeloma, and of textile manufacturing in cancer of the colon and prostate.

Project Description

Objectives: To identify and evaluate groups at high risk of developing cancer because of carcinogenic materials in the work environment through analytic investigations of various occupational groups.

Methods Employed: Cancer patterns are determined through long-term follow-up of persons employed in specific plants, industries, and occupations. Follow-up resources for cohort studies include the Social Security Administration, Office of Personnel Management, state motor vehicle bureaus, state vital statistics offices, city directories, and the post offices. The cancer experience of study groups is usually compared to that of the general population (geographic specific, if possible). In some instances, comparisons are made with other industrial populations. Proportionate mortality studies are conducted when population data are unavailable. Case-control studies of persons with particular cancers are carried out in certain geographic areas where industries or occupations of interest are concentrated. Occupational, demographic, and other information may be obtained on study subjects by personal interview or from available employment records.

Major Findings: The attached list (Appendix A) shows occupational cancer studies in progress. Findings from completed studies include:

1. A retrospective cohort study of male chemists employed at a chemical company found the total number of cancer cases and cancer deaths to be fewer than expected. Chemists did appear to be at slightly elevated risk from melanoma, and cancer of the colon and prostate.

2. Disability between 1959 and 1977 among chemists was compared to other salaried employees from the company. Chemists experienced fewer disabilities from absences (lasting eight or more days), benign and unspecified neoplasia, heart disease, peptic ulcer, and diseases of the urinary system, bones and joints, and skin than other employees. Exposure misclassification and socioeconomic status confounding may have contributed to these deficits.

3. Existing systems widely used by epidemiologists to classify study subjects according to occupational exposures possess weaknesses not evident to many investigators. The strengths and weaknesses of these systems were evaluated and discussed focusing on epidemiologic needs.

4. An occupation and exposure linkage system was developed to facilitate categorization of study subjects by exposure. Classification systems based on occupation and industry currently available may misclassify subjects and reduce or obscure associations between specific exposures and disease.

5. Analysis of cause of death among professional artists revealed an excess of leukemia and cancers of the bladder, kidney, brain, colon, and prostate among males. The excesses for leukemia and cancer of the bladder were particularly striking among painters, while the excess for prostate cancer was limited to sculptors. Among females (particularly female

painters), higher than expected proportions of cancers of the rectum, lung, and breast were observed. These excesses may be due to pigments and dyes, metal fumes, and dusts used by professional artists in their work.

6. A proportionate mortality investigation of cancer among members of the International Molders and Allied Workers Union noted an excess of lung cancers. In a follow-up of this finding, a nested case-control study found an increased risk for lung cancer among younger ($\leq$65 years at death) workers in iron foundries, but not among workers from steel and nonferrous foundries.

7. A case-control study among rubber workers was conducted to evaluate the role of solvents and complex hydrocarbons in the origin of brain cancer. Although industrial hygiene assessments of individual exposures were not available, the risk of brain cancer was not unusual in any of the occupational categories evaluated.

8. A computer program was developed to link pollutant levels to individual study subjects. The method allows multiple univariate observations on pollutant levels to be transformed into a single multivariate observation on each subject.

9. A case-control study of bladder cancer among Wisconsin farmers (1968-1976) revealed no excess risk among farmers in general or dairy farmers in particular. A similar study of leukemia, however, showed increased risks for farmers born more recently, dying at younger ages, and from counties where fertilizer usage and dairy production were heavy. The risks of leukemia among study subjects specifically noted as dairy farmers were similar in pattern and magnitude to those among farmers in general.

10. An evaluation of the characteristics of smokers, ex-smokers, and nonsmokers in a working population uncovered few differences among the groups. This lack of variation among these groups provides little support for the constitutional hypothesis of smoking risk. The associations, however, with spouse smoking habits, on-the-job physical activity, and Type A behavior may be useful in designing smoking cessation programs.

11. A proportionate mortality study of cancer among petrochemical workers in Texas City, Texas revealed excesses of deaths from cancers of the skin and brain from one company. Additional deaths from brain cancer have been identified among workers at this company who were not eligible for inclusion in this study.

12. When mortality data from an earlier report were supplemented with records on retired members of the Oil, Chemical, and Atomic Workers Union, excess mortality from certain cancers was observed. Proportionate mortality ratios were high for leukemia, multiple myeloma, and lymphoma, particularly among retired workers. Relative frequencies for brain tumor deaths were significantly elevated among active workers and slightly elevated among retirees. Case-control studies were designed to further evaluate this occupational association by summarizing information from work histories.

Although the numbers are small, preliminary results showed a larger percentage of cases than controls in the occupations involved in moving crude oil and refinery products from one point to another. In addition, the mean length of employment in the motor oil category (involves second-step refinery operations such as the manufacture of lubricating oils, paraffin, and some solvents) was much longer for the cases than the controls.

13. Although the irritant effects of formaldehyde are well known, recent laboratory experiments raised serious concerns regarding its carcinogenicity. To evaluate the potential hazards among humans, a proportionate mortality study of embalmers was undertaken. Increased frequencies of death from cancer of the skin, kidney, and brain were observed, particularly among those licensed for more than 35 years, or those who were first licensed before age 30. Although embalming fluids contain chemicals other than formaldehyde, these excesses suggest a need for additional studies to further clarify the potential risk associated with formaldehyde exposure.

14. Other activities by the staff included: a) reviewing methodologic issues of risk assessment using data from epidemiologic studies on occupational or industrial groups; b) serving on review groups such as the Federal Panel on Formaldehyde (charged with reviewing laboratory and epidemiologic data to assess the potential health risk posed by formaldehyde exposure), NCI Group to Evaluate the OSHA Candidate List of Chemicals for Possible Regulation, and Threshold Limit-Value Committee of the American College of Governmental Industrial Hygienists; and c) providing advice and guidance to other agencies and organizations regarding the design and conduct of occupational studies.

Significance to Biomedical Research and the Program of the Institute: Studies of the cancer experience of working populations have provided much of the information known about chemical carcinogenesis in man. Occupational groups may be regarded as indicators in evaluating possible hazards to the general population because exposures are often heavy and well defined.

Proposed Course: An increasing number of industrial populations will require epidemiologic investigation as leads are developed from clinical and laboratory observations. New studies are being initiated to meet this need. In addition, findings from completed studies suggest areas and methodologies where further research is needed to identify more accurately carcinogenic agents associated with the workplace and to better quantify the cancer risks.

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Appendix A.

04/16/81

National Cancer Institute

Environmental Epidemiology Branch

Occupational Studies Section

TITLE AND INVESTIGATOR	SUSPECT EXPOSURES	DATA SOURCE	METHODOLOGY
I. STUDIES UNDERWAY (Investigator)			
1. Dry cleaners (Blair)	Petroleum solvents, benzene, carbon tetrachloride, TCE, PERC	Membership records of a single Union local in St. Louis (N=10,000 persons)	Cohort Mortality Study (1940's to present)
2. Men serving in chemical processing companies during World War II (Fraumeni)	Tetrachloroethane	Military records (N=7,000 men)	Cohort Mortality Study (1940's to present)
3. Lab personnel employed at Ft. Detrick, MD (Mason)	Hyperimmunization	Personnel records at Ft. Detrick (N=3,000 persons)	Cohort Mortality Study (1945 to present)
4. Structural pest control operators (Blair)	Pesticides of various kinds	State licensing records in Florida (N=4,411 persons)	Cohort Mortality Study (1965 to 1978)
5. Tobacco workers (Blair)	Tobacco dust, pesticide residues	Union death records (N=3,000)	Proportionate Mortality Study (1957 to 1978)
6. Fur workers (Walrath)	Dyes, sawdust	Union pensioners' records (N=1,200)	Cohort Mortality Study (1950 to present)
7. Embalmers (Walrath)	Formaldehyde	Licensing records from California (N=1,000)	Proportionate Mortality Study (1950 to present)
8. Textile workers (Hoar)	Dyes, finishing compounds	Death certificates from South Carolina (N=2,110)	Case-control Study of prostate cancer (1968 to 1976)
9. Carpet workers (Hoar)	Dyes, finishing compounds	Death certificates from South Carolina (N=1,650)	Case-control Study of large bowel cancer (1968 to 1976)
10. Veterinarians (Blair)	X-rays, animal viruses, chemicals	Death notices in monthly issues of JAVMA (N=3,000 deaths)	Proportionate Mortality Study (1950 to 1966)

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TITLE AND INVESTIGATOR	SUSPECT EXPOSURES	DATA SOURCE	METHODOLOGY
11. Welders (Thomas)	Chromium- and nickel-containing welding fumes	Employment records of one or more companies (N=3,000)	Cohort Mortality Study (1952 to present)
12. Chemical plant workers (Blair)	Benzene and a variety of other chemicals used to make detergent alkylates	Employment records of a single plant (N=265 persons)	Cohort Mortality Study (1940's to present)
13. Smoking by occupation and industry (Walrath)	Occupations and industries in 1950 Census	Dorn Study of U.S. Veterans (N=300,000)	Cohort Mortality Study (1954 to 1979)
14. Male chemists (Walrath)	Chemicals	Membership records of ACS (N=50,000)	Cohort Mortality Study (1966 to present)
15. Female chemists (Walrath)	Chemicals	Membership records of ACS (N=600)	Proportionate Mortality Study (1948 to present)
16. Cosmetologists (Walrath)	Hair dyes, hair sprays	State licensing records (N=12,000)	Case-control Study (1948 to present)
17. Cutting oils (Thomas)	Cutting oil mists	Company records (N=23,000)	Cohort Mortality Study further follow-up (1967 to 1979)
18. Paint, enamel, lacquer, varnish manufacturing workers (Miller)	Pigments, solvents, various resins, formaldehyde	Union records of active and retired workers (N=400)	Proportionate Mortality Study (1955 to present)
19. Pigments manufacturing workers (Miller)	Various metallic oxides	Union records of active and retired workers (N=400)	Proportionate Mortality Study (1955 to present)
20. Association of parental occupation with childhood cancer cases (Spirtas)	Radiation, hydrocarbons	British National Survey	Case-control Study on all British cancers using individually matched controls (1953 to present)
21. Latency in angiosarcoma cases (Spirtas)	Vinyl chloride, thorotrast	NIOSH Registry and published literature	Regression modeling of case histories (1955 to present)
22. Non-ferrous smelter workers (Blot)	Arsenic, sulfur dioxide	Employment records of a single company (N=5,412 men)	Cohort Mortality Study (1963 to present)
23. Taconite mine and iron ore pellet facility (Cantor)	Fibrous materials	Employment records of a single company (N=9,500 persons)	Cohort Mortality Study (1955 to present)

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TITLE AND INVESTIGATOR	SUSPECT EXPOSURES	DATA SOURCE	METHODOLOGY
24. Jewelry workers (Blair)	Metals, solvents	Membership records of a Union local in New York (N=15,000)	Cohort Mortality Study (1945 to 1965)
25. Occupational study of myeloma in Nebraska (Blair)	Various farming-related exposures	Death certificates from Nebraska (N=600)	Case-control Study examining occupational statements on death certificates (1968 to 1974)
26. Lung cancer and occupation (Hoover)	Lumbering and sawmills, shipyards	Kaiser Health Plan, Portland, Oregon	Case-control Study using smoking and occupational information from a standardized questionnaire (1970 to 1979)
27. Reproductive outcomes among dry cleaners (Blair)	Tetrachloroethylene, petroleum solvents	Union membership records and interviews (N=600)	Collaborative Case-control Study with NIEHS (1970 to 1979)
28. Barge and ship inspectors (Blair)	Chemicals transported by ships	Employment records of the Coast Guard (N=5,000)	Cohort Mortality Study (1943 to 1970)
29. Hematite miners (Lubin)	Iron oxide	Employment records of several companies in Minnesota (N=11,000)	Cohort Mortality Study (1910 to 1965)
30. Petrochemical workers (Thomas)	Petroleum products	Union and company records (N=400)	Case-control Study of lung, brain, and stomach cancer (1947 to 1977)
31. Photographers (Miller)	Developers, fixatives	Death notices (N=250)	Proportionate Mortality Study (1963 to 1978)
32. U.S. Department of Agriculture employees (Blair)	Pesticides	Death benefit files from the Office of Personnel Management (N=14,000)	Proportionate Mortality Study (1945 to present)
33. Shipyard workers (Grauman)	Asbestos, metal fumes, and dusts	Employment records of the Coast Guard (N=5,000)	Cohort Mortality Study (1950 to 1964)
34. Medical technologists (Grauman)	Formaldehyde, fixatives, stains	Licensing records from California (N=15,000)	Cohort Mortality Study (1948 to present)
35. Pesticide poisoning (Walrath)	Pesticides	Reports of pesticide poisonings from California (N=10,000)	Cohort Mortality Study (1972 to present)
36. Beekeepers (Cantor)	Hyperimmunization	USDA and Association records (N=8,000)	Cohort Mortality Study (1960 to 1979)

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	TITLE AND INVESTIGATOR	SUSPECT EXPOSURES	DATA SOURCE	METHODOLOGY
37.	Workers in industries producing and using formaldehyde (Blair)	Formaldehyde	Company records (N=8,000)	Cohort Mortality Study (1940 to 1965)
38.	X-ray technologists (Boice)	Radiation	Registry records (N=170,000)	Cohort Mortality Study (1926 to present)
39.	Leukemia and lymphoma among farmers (Blair)	Solvents, pesticides, fuels	Hospital records and interviews (N=600 cases; 1,200 controls)	Case-control Study (1980 to 1982)
40.	Furniture workers (Blair)	Wood dusts, solvents, varnishes and paints	Union records (N=30,000)	Cohort Mortality Study (1945 to 1965)
41.	Corn wet-milling workers (Thomas)	SO_2, pesticide residues	Union records (N=800)	Proportionate Mortality Study of active and retired workers (47 to 1977)
42.	Pharmaceutical workers (Thomas)	Pharmaceuticals and biologics	Union records (N=500)	Proportionate Mortality Study (1947 to present)
43.	Coast Guard (Winn)	Exposures encountered in various job categories	Disability and Personnel files (N=600)	Case-control Study (1958 to 1979)
44.	Herbicides and lymphomas and soft-tissue sarcomas (Hoar)	Herbicides	Interview study of next-of-kin (N=200 cases of each tumor; 3-times as many controls)	Case-control Study (1975 to 1979)
45.	Anatomists (Blair)	Formaldehyde and other preservatives	Archives of the Association of American Anatomists held by Brown University (N=3,000)	Cohort Mortality Study (1920 to present)
46.	Histologic technicians (Grauman)	Xylene, formaldehyde, chloroform stains	Certification records from the American Society of Clinical Pathologists.	Cohort Mortality Study (1947 to 1965)
47.	Non-Hodgkin's lymphoma in farmers (Cantor)	Pesticides, herbicides, bovine leucosis virus	Death records from Wisconsin (N=780)	Case-control Death Certificate Study (1968 to 1976)
48.	Plumbers and pipefitters (Cantor)	Welding fumes, solvents, asbestos	Union mortality records from California (N=7,500)	Proportionate Mortality Study (1960 to 1979)

	TITLE AND INVESTIGATOR	SUSPECT EXPOSURES	DATA SOURCE	METHODOLOGY
49.	Structural pest control operators (Cantor)	Various pesticides	State licensing records from California (N=4,000)	Cohort Mortality Study (1955 to present)
50.	Aerial applicators (Cantor)	Various pesticides	FAA records (N=11,000)	Cohort Mortality Study (1965 to present)
51.	Shoe workers (Walrath)	Dyes solvents, leather, dust	Obituary notices (N=4,000)	Proportionate Mortality Study (1960 to 1979)
52.	Brain cancer and occupation (Thomas)	Petroleum, petrochemicals	Death certificates from areas with a heavy concentration of the chemical industry (N=1,200)	Case-control Next-of-Kin Interview Study
53.	Sanitary-ware workers (Thomas)	Talc and wallastonite	Company records	Cohort Mortality Study
54.	Mesothelioma linking SEER and VA with SSA (Spirtas)	Asbestos, fibrous dusts	SEER Registries, SSA employment history files, VA pathology files	Case-control Study (1935 to present)
55.	Pharmaceutical workers (Thomas)	Multiple chemical exposures	Union records	Cohort Mortality Study of workers at a single plant
56.	Solvent exposures in airplane maintenance (Spirtas)	Tetrachloroethylene, chloroform, zinc chromate	Employment records of the Air Force (N=20,000)	Cohort Mortality Study (1952 to present)
57.	Occupation and multiple myeloma in Wisconsin (Cantor)	Farm-related exposures	Death records from Wisconsin	Case-control Study Death Certificate Study (1968 to 1976)

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Appendix B. Contracts in Support of this Project

EQUIFAX SERVICES, INC. (NO1-CP-11010)

Title: Special Support Services for Tracing Individuals.

Current Funding Level: $565,282.

Man Years: 1.25

Objectives: To trace individuals from cohorts being studied by investigators in the Environmental Epidemiology Branch, as well as in other program branches, and to determine their vital status, place of death if applicable, and last known address if living. All individuals being traced had an exposure to a substance, agent, or other factor under investigation, usually many years before. Death certificates will be obtained by the Branch.

Methods Employed: The sequential tracing steps followed by Equifax were to matching cohort names, with other identifiers if available, against credit bureau files (40% of the contract effort), against motor vehicle bureau records (25% of effort); against own company files in various geographical locations (15% of effort); to check telephone and other directories, and telephone inquiries to close neighbors (15% of effort); and finally, personal checking by Equifax field offices (5% of effort).

Major Finding: Some cohorts traced under this contract had environmental exposures to suspect carcinogens as far back as 1930. In addition to name changes in women, there have been address changes and mortality among most cohorts. Success in tracing by this company has usually been under 50%, and often under 25%, the cost increasing with difficulty. Women in cohorts which go back to 1930 have proven to be the most difficult to trace, and those from 1940 onward not as difficult. Tracing by credit bureaus outside Equifax's own system is often difficult because they may wish to have address and zip codes, neither of which may be known in advance. Equifax field offices were the least useful and the most expensive to utilize. Examples of cohorts to be traced were: 3,946 dry cleaning workers in St. Louis, 1930-1980; 3,014 jewelry manufacturing workers in New York, 1930-1980; 350 cervical cancer patients, 1930-1954, in several eastern states; and 728 former TB patients in Massachusetts, 1930-1954.

Proposed Course: At the end of one year, it will be determined whether this full-time tracing contract should be continued, or whether it might be much less expensive and more effective to use another tracing mechanism, such as under purchase orders or by incorporating the tracing effort into an ongoing support contract as a part-time effort. Credit bureaus and motor vehicle bureaus checks can be purchased separately, if necessary.

Date Contract Initiated: December 8, 1980.

WESTAT, INC. (NO1-CP-91034)

Title: Support Services for Occupational Studies.

Current Annual Level: $753,979.

Man Years: Eleven.

Contract's Project Director: Frank Moran.

Objectives: To provide technical, managerial, and clerical support for occupational studies conducted by the Environmental Epidemiology Branch.

Methods Employed: Westat, Inc. provides personnel to assist NCI in data collection for cohort, proportionate mortality, and case-control studies. This includes developing abstracting forms and questionnaires, administering these instruments, maintaining quality control of data collection, tracing study subjects, editing and updating data, and preparing final data files.

Major Contributions: This contract provides support for some 25 different projects. Significant contributions were to items 5, 6, 9, 12, and 13 under Major Findings for the Intramural Research Project "Studies of Occupational Cancer."

Proposed Course: Expiration date for this contract is September 29, 1983 and should continue to that date in accordance with above methodology.

Date Contract Initiated: September 29, 1979.

CONTRACTOR TO BE SELECTED (NO1-CP-11006)

Title: Support Services for a Mortality Study of Workers Exposed to Formaldehyde.

Estimated Funding Level: $200,000.

Estimated Man Years: Five.

Objectives: To provide technical and managerial support for data collection for a cohort mortality study of formaldehyde-exposed workers.

Methods to be Employed: The contractor will assemble a cohort of 5,000 to 10,000 workers from 10-20 companies whose workers have had exposure to formaldehyde. Data will be abstracted from personnel records, coded, edited, and entered in the computer. All study subjects will be traced to determine their present-day vital status.

Estimated Initiation Date: June, 1981.

CONTRACTOR TO BE SELECTED (NCI-CP-FS-11019)

Title: Support Services for a Case-Control Study of Brain Cancer and Occupation.

Estimated Funding Level: $200,000

Estimated Man Years: Three.

Objectives: This contract is to provide NCI with the necessary field support to conduct a case-control interview study of brain cancers to evaluate the role of occupation (particularly employment in the petroleum industry) and other factors in the origin of this cancer.

Methods to be Employed: Six hundred cases and 600 controls among adult males will be selected from geographic areas where at least 10% of the males are employed in the petroleum industry. Cases and controls will be identified from mortality records at State Vital Records offices. Next-of-kin of cases and controls will be interviewed to determine salient factors regarding the the origin of this tumor.

Estimated Initiation Date: August, 1981

CONTRACTOR TO BE SELECTED (P00057)

Title: Mortality among Airplane Maintenance Workers.

Estimated Funding Level: $225,000 to be funded by DOD/U.S. Air Force.

Estimated Man Years: Four.

Objectives: To compare the mortality experience of workers exposed to solvents and other hazardous substances with internal and external control groups.

Methods to be Employed: A cohort of 15,000-20,000 civilian workers at the Hill Air Force Base maintenance facility in Utah during 1952-57 will be assembled from personnel records. Occupational exposures will be estimated from job titles and departments. The present-day vital status of each member will be ascertained and cause-specific mortality rates calculated for comparison with expected rates from the general U.S. population and unexposed civilian workers at the air base.

Estimated Initiation Date: August, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04481-05 EEB

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)
Studies of Radiation-Induced Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Co-PI:	Charles E. Land	Health Statistician	EEB NCI
Co-PI:	John D. Boice, Jr.	Epidemiologist	EEB NCI
Others:	Daniel A. Hoffman	Epidemiologist	EEB NCI
	Elaine Ron	Visiting Scientist	EEB NCI
	Ruth A. Kleinerman	Epidemiologist	EEB NCI
	Diane J. Mundt	Epidemiologist	EEB NCI
	Gilbert W. Beebe	Health Statistician	CEB NCI
	William J. Blot	Health Statistician	EEB NCI
	Mark H. Greene	Clinical Investigator	EEB NCI
	Betty J. Stone	Mathematician	EEB NCI
	Rochelle Curtis	Statistician	BB NCI
	Linda Pottern	Epidemiologist	EEB NCI
	Joseph Scotto	Demographer	BB NCI

COOPERATING UNITS (if any)
Clinical Epidemiology Branch, FSS,DCCP,NCI
Radiation Effects Research Foundation
Bureau of Radiological Health, FDA
Medical Follow-up Agency
Biometry Branch, FSS,DCCP,NCI
Department of Energy

LAB/BRANCH
Environmental Epidemiology Branch

SECTION
Radiation Studies Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
7.0	6.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives of this project are to examine cancer incidence and mortality among populations exposed to ionizing and non-ionizing radiation; to characterize the risk of radiation-induced cancer in terms of tissues at risk, dose response, radiation quality, temporal distribution of dose, time since exposure, age at exposure and at observation for risk, and possible modifying influences of other environmental and host factors; and, in particular, to quantify excess cancer risk at low dose levels. Groups studied include the Japanese A-bomb survivors, several large populations with documented therapeutic, diagnostic, and occupational exposures to ionizing radiation, and resident populations of SEER reporting areas with different ambient levels of solar UV radiation. Other biological effects, such as chromosomal abnormalities in circulating lymphocytes, birth defects, benign tumors, and other non-neoplastic changes, are studied for possible insights into radiation carcinogenesis. Close liaison is maintained with experimental scientists at NCI and other government laboratories concerned with radiation carcinogenesis. Project members serve on committees and task forces advising the government at various levels on health effects of ionizing radiation, and formulating government responses to current problems in this area.

Project Description

Objectives: (1) To plan and conduct studies of cancer risk and related health outcomes in populations exposed to ionizing radiation (e.g., x-ray, gamma ray, neutrons, alpha and beta particles) and nonionizing radiation (e.g., ultraviolet light); (2) to evaluate excess cancer risk in exposed populations in terms of tissues at risk, amount, quality, and temporal and spatial distribution of radiation dose, latency, and possible modifying influences of host factors (e.g., age, sex, hormonal status) and external factors (e.g., drugs, other carcinogens); (3) in particular, to improve estimates of cancer risks associated with exposure to low doses of ionizing radiation; (4) to examine possible analogues of radiation carcinogenesis in man, such as the induction by radiation of benign tumors and cytogenetic abnormalities in man, or cancer and other effects in experimental systems, for insights into mechanisms of radiation carcinogenesis that may also result in improved theoretical models for the estimation of risk; and (5) to advise and collaborate with other government agencies involved in radiation research and the control of risks from activities involving radiation exposure.

Methods Employed: 1. Studies of Japanese A-Bomb survivors
Investigations based on the Life Span Study sample of 82,000 A-bomb survivors and 26,000 nonexposed controls are carried out at the Radiation Effects Research Foundation (RERF) in Hiroshima and Nagasaki, Japan. All studies involving new or unpublished data are collaborative, and include investigators from NCI, RERF, and outside organizations as required; collaboration is facilitated by personnel exchanges between RERF and NCI. Protocols for collaborative studies undergo formal review at RERF and NCI.

a. A case-control study based on interviews of living breast cancer cases and matched controls in the LSS sample, and available records for deceased cases and their controls, has continued in Hiroshima and Nagasaki. The study was designed to investigate possible interactive effects of radiation and other environmental and host factors in the etiology of female breast cancer.

b. A second case-control study, having similar aims with respect to the etiology of lung cancer, was initiated.

c. A cohort-based study of breast cancer incidence in the LSS sample, covering the period 1950-1980, was initiated.

d. Following up suggestive findings from a 1979 US-Japan pathology review of diagnostic materials for breast cancer in the LSS sample, a pathology study was initiated of breast tissue samples from breast cancer cases and autopsy controls, with respect to possible non-neoplastic changes related to radiation dose and breast cancer risk.

e. An analysis of pathology material from an earlier breast cancer series, covering the period 1950-1969, was completed and published as the final report in the Atomic Bomb Casualty Commission Technical Report series.

f. Existing records of early post-bomb mortality were evaluated to investigate the possibility that radiation effects on immunity might have biased current estimates of cancer risk as a function of radiation dose, through differential mortality from infectious disease.

g. Published data on cancer mortality and incidence through 1974, acute radiation effects, chromosome aberration frequencies, and developmental abnormalities were analyzed with respect to gamma and neutron dose as determined by two separate dosimetries, in an effort to clarify the biological implications of current uncertainties in radiation dose estimates.

h. A case-control study (with RERF and the Department of Community Medicine, University of Southern California) was initiated, based on hormonal assays of stored blood samples. These samples, obtained prior to cancer diagnosis, are to be selected from LSS sample members diagnosed with cancers of the breast, prostate, and thyroid, and from matched controls.

i. A collaborative study was initiated involving parallel dose-response analyses of thyroid cancer incidence data from several different series, in collaboration with investigators from RERF, Tel Aviv University, New York University, and Michael Reese Hospital (Chicago). The study is modelled after a similar collaborative approach to breast cancer incidence among A-bomb survivors and patients receiving medical radiation during treatment for TB and acute postpartum mastitis (see publication list).

2. Studies of patients with histories of medical exposure to radiation.

a. The follow-up of approximately 15,000 persons exposed to multiple flouroscopic chest examinations in conjunction with pneumothorax treatment of tuberculosis is continuing. The TB-fluoroscopy studies are conducted in collaboration with Harvard University and Yale University, and all TB patients treated between 1930-1954 in Massachusetts and Connecticut are being studied. The risk of radiogenic breast cancer in older women, and lung cancer and leukemia in men and women is being evaluated. A pilot telephone interview case-control study of breast cancer has been initiated to evaluate the interaction of radiation and host factors. These studies are of particular public health importance since the exposures were to low doses of radiation, repeated every few weeks for many years.

b. A large international study of 30,000 women treated for cervical cancer in 30 radiotherapy centers in 9 countries is continuing. In addition to the evaluation of radiogenic leukemia, the study has been expanded to evaluate the risk of developing solid tumors following relatively low-dose irradiation. Doses to body sites outside the pelvis are low (1-100 rads) and can be accurately determined. In addition to this clinical sample, the study has been expanded to include cervical cancer patients reported in 20 cancer registries across the world. Approximately 200,000 women are under study making this the largest radiation investigation yet conducted. Case-control studies within the various cohorts are being conducted, in addition to straightforward cohort evaluations. Dosimetry, pathology, questionnaire, and blood studies are ongoing.

c. A study of 3,300 children treated for enlarged tonsils with irradiation or surgery has continued in collaboration with the Children's Hospital in Boston. Physical examinations have been initiated for both irradiated and surgical patients to more accurately determine the risk of thyroid nodular disease, and to adjust for the potential detection bias in previous studies which only screened radiation-exposed persons. The effect of small occult tumors, found only during special screening, on risk estimates will be investigated. Blood studies will determine radiation effects on the parathyroid and thyroid, and chromosome aberrations in circulating lymphocytes will be evaluated.

d. In collaboration with the Department of Energy-supported Cytogenetics Laboratory at the Oak Ridge Associated Universities, an investigation was initiated in which chromosome aberration frequencies will be ascertained for circulating lymphocytes in radiation-exposed and control subjects from three different patient populations under study as described in items a-c above. The study is intended to calibrate the technique of chromosomal analysis as a biological dosimeter for partial-body radiation exposures, and to explore the analogy between the induction of chromosomal aberrations and carcinogenesis.

e. Using the resources of pre-paid health plans in California and Oregon (Kaiser-Permanente) cases of leukemia and lymphoma and controls are being identified and life histories of diagnostic x-ray exposures are being obtained. Approximately 2,000 cases are being studies and the association with diagnostic x-rays evaluated.

f. Record linkage of data tapes of the Connecticut Tumor Registry and Twin Registry has been initiated to evaluate the risk of developing childhood cancer in twins. Controls are being selected and hospital records searched to evaluate the risk of prenatal x-ray exposure. A similar collaborative study is planned in Sweden to obtain a larger sample size.

g. A case-control interview study of all thyroid cancer cases diagnosed between 1978-1980 in Connecticut has been initiated. Approximately 200 persons with thyroid cancer and 400 population controls will be interviewed to evaluate the influence of radiation, drug and diet on thyroid cancer risk.

h. The Surveillance Epidemiology and End Results (SEER) registries were used to identify second primary cancers in persons treated for Hodgkin's disease, cervical cancer, prostate cancer, testicular cancer, and thyroid cancer. In addition to evaluating the carcinogenicty of alkylating chemotherapeutic agents, radiation treatments are being evaluated as a possible cause of second primary cancers. Cohort and case-control studies are being conducted.

i. Patients receiving whole body irradiation at the National Cancer Institute for non-Hodgkin's lymphoma are being evaluated for excess leukemia risk.

j. A case-control study of over 200 children who developed second primary cancers following treatments for childhood cancer is continuing at 13 childhood cancer centers in 6 countries.

k. A collaborative study is continuing with the Chaim Sheba Medical Center, Israel, to evaluate the risk of cancer in 10,000 children exposed to x-rays during thr treatment of ringworm of the scalp, and 15,000 comparison persons. The methods are unique in that all pathology records of Israeli hospitals are being searched to identify tumors. Analysis of existing data has been initiated for cancer incidence and mortality.

l. A case-control study of salivary gland cancer using the resources of the Armed Forces Institute of Pathology was initiated.

m. The safety and efficacy of photochemotherapy (oral methoxsalen and UV-A radiation) for psoriasis were evaluated in a 15 hospital centers clinical trial involving 1,125 patients.

n. The risk of malignancies following therapeutic doses of 131-I in the treatment of hyperthyroidism has been evaluated in collaboration with the Bureau of Radiological Health (FDA). Mortality and morbidity analysis were completed on a USPHS study of 36,000 thyrotoxicosis patients. The detailed follow-up at one participating clinic has suggested the desirability to expand the follow-up of these patients, and this has been initiated. A study of second cancers following 131-I treatment for thyroid cancer at these same hospitals was nearly completed.

3. Studies of patients with histories of occupational exposure to ionizing radiation.

a. A successful feasibility study was completed and a full scale investigation was begun to evaluate the risk of cancer among 170,000 x-ray technologists identified from registry records for the years 1926-1980. This is a collaborative study with the University of Minnesota.

b. Sputum cytology data on uranium miners, controlling for radiation exposure and cigarette smoking, were analyzed. This has been a collaborative effort with NIOSH and Dr. Geno Saccomanno, a private physician of Grand Junction, Colorado.

4. Population studies of environmental radiation exposures.

a. Regions in the Western U.S. with uranium mill tailing deposits were identified and correlations with county cancer mortality data made.

b. In collaboration with the Biometry Branch, a study was continued to clarify the role of solar UV-radiation in the development of non-melanoma skin cancer by means of a demographic survey using SEER Tumor Registries, and a case-control study to clarify the influence of various host and environmental co-factors.

5. Investigations into statistical methodologies for estimation of risk

a. Several expository articles were written about methodological problems of estimating cancer risk from low doses of ionizing radiation, including the effect on statistical power of general models reflecting the current state of knowledge about mechanisms of radiation carcinogenesis.

b. Formal Bayesian procedures, by which prior and experimental information about dose-response functions can be used in estimating low-dose risk from high-dose epidemiological data, are being investigated in collaboration with Oregon State University.

6. Consultant Activities and Services on Expert Committees. The co-Principal Investigators have served as consultants or committee members for the National Council on Radiation Protection and Measurements, the Department of Energy, the Department of Defense, the Environmental Protection Agency, the National Academy of Sciences-National Research Councils Advisory Committee on the Biological Effects of Ionizing Radiation, the DHHS subcommittee to coordinate federal radiation activities, the American Cancer Society National Committee on Cancer Prevention and Detection, and the National Academy of Sciences Panel on Hiroshima/Nagasaki Occupation Forces.

7. Symposium on Radiation Carcinogenesis. Although delayed for several years, arrangements for a symposium on radiation carcinogenesis have continued. The purpose will be to allow principal investigators, who have conducted major human epidemiologic studies of radiation and cancer, to summarize their findings, present current work, and suggest areas for future research. The implication of radiation studies to models of carcinogenesis in general will be stressed.

8. Review Papers. Several review papers concerning health effects following exposure to ionizing radiation were written, including a general overview, a review of cancer following medical irradiation, the epidemiology of radiogenic bone cancer, the effect of radiation on the immune system, the statistical aspects of estimating cancer risks from low doses of ionizing radiation, the epidemiologic issues concerning low-dose radiation studies, the implications of radiogenic breast cancer studies for models of human carcinogenesis, and the long-term effects of radiation upon the human fetus.

Major Findings: 1. The dose-response function for radiogenic breast cancer appears to be linear. This conclusion follows from three separate results. First, the fitted dose-response functions for the Hiroshima and Nagasaki A-bomb survivors are similar, suggesting equivalence between neutrons and gamma rays with respect to their carcinogenic effects on breast tissue. This result is most consistent with linearity of effect according to current radiobiological theory. Second, fitted dose-response functions for A-bomb survivors, mastitis patients, and TB-fluoroscopy patients are consistent with linearity. Finally, all three populations have similar age groups. This result suggests that breast cancer risk is independent of dose fractionation, a result consistent with linearity and inconsistent with marked curvilinearity.

2. Further analysis of the studies of TB patients who had multiple fluoroscopic examinations of the chest, mastitis patients given radiotherapy, and A-bomb survivors, suggest that the risk for radiogenic breast cancer is greatest for persons exposed as adolescents, although exposure at all ages carries some risk. Direct evidence of radiation risk at doses under 50 rads is apparent among A-bomb survivors. The interval between exposure and clinical appearance of radiogenic breast cancer may be mediated by hormonal or other age-related factors, but is unrelated to dose. It is as yet impossible to determine whether an absolute or relative risk model more correctly describes the relationship between spontaneous and radiation-induced breast cancer.

3. Radiation-related breast cancers appear morphologically similar to other breast cancers occurring in women of comparable age, according to an evaluation of pathological materials from breast cancer cases diagnosed among A-bomb survivors during the period 1950-1969.

4. Mortality from infectious disease among A-bomb survivors during the periods 1946-1950 and 1950-1966 appears to be unrelated to radiation

dose, thus failing to confirm speculations that the A-bomb survivor data might be biased because of differential mortality resulting from radiation-induced immune deficiencies. Similar results were found in an analysis of published material on mortality among British patients treated by radiation for ankylosing spondylitis.

5. Hiroshima-Nagasaki differences in dose response for certain radiation effects, which have been ascribed to differences in the quality of radiation received from the A-bombs dropped on the two cities, persist and are difficult to explain according to a proposed new dosimetry in which city differences in radiation quality are much less marked. The A-bomb survivor data have less relevance to questions about the shapes of dose-response curves according to the proposed new dosimetry.

6. Existing (and forseeable) epidemiological data are unlikely to yield precise estimates of risk from low-dose radiation exposures unless arbitrary assumptions are made that severely restrict the possible forms taken by the fitted dose-response function, or unless relevant information can be incorporated from experimental data. Bayesian methods allow uncertainties of assumptions and variability of experimental data to be taken into account when incorporating such information into curve-fitting analyses of epidemiological dose-response data.

7. The second mail questionnaire follow-up of women who received multiple chest fluoroscopies in conjunction with pneumothorax treatment of tuberculosis reaffirms that repeated relatively low radiation doses pose some future risk of breast cancer, that the risk may be cumulative, and that a woman's lifetime risk of breast cancer is likely determined in large part during early adult life. Exposures around the menarche and during first pregnancy appear especially hazardous, so that proliferating breast tissue may be particularly sensitive to the carcinogenic effects of radiation. Nulliparous women appear to be at higher radiation risk than women who have had children.

8. Additional studies have been conducted on tuberculosis patients in Massachusetts exposed to repeated fluoroscopic chest examinations. The estimation of radiation doses for the breast, lung, stomach, pancreas, and active bone marrow was refined and a mortality analysis performed. No excess of total cancer deaths, leukemia, lung cancer, or lymphoma was seen among fluoroscopically examined women. The mortality analysis is meaningful to the extent that upper levels of excess radiation risk might be excluded: 12 deaths per million women/yr/rad for leukemia, 3.5 deaths per million women yr/rad for lung cancer, 4.8 deaths per million women/yr/rad for lymphoma. These findings indicate that the carcinogenic effect of multiple low-dose x-ray exposures is unlikely to be greater than currently assumed.

9. A 10-year international radiation study of 30,000 cervical cancer patients, treated in 9 countries and followed clinically with blood studies, failed to observe any excess leukemia, although the bone marrow doses were large and a substantial excess was predicted. Among 28,000 women treated with intracavitary radium, external radiation or both, 13

cases of leukemia were observed against 15.5 expected. A 2-fold risk could be exlcuded but a 1.4-fold risk remained possible. In absolute terms, risks larger than 0.1 leukemia cases per million women/yr/rad could be excluded. The absence of an effect suggests that (a) high doses to small volumes of bone marrow may cause substantial cell-killing and minimal cell transformation; (b) patients may not have been followed long enough to observe an effect if the latent period for older persons is longer than for younger individuals; and/or (c) women may be less sensitive than men to radiogenic leukemia. A deficit of chronic lymphocytic leukemia was observed suggesting that radiation may actually protect against the development of this disease. A significant deficit of multiple myeloma was also found. Doses to body sites outside the pelyic area from radium are low (1-100 rad) and can be accurately determined. This study has now been extended to evaluate the risk of excess solid tumors following low-level exposures.

10. Subsequent cancers were examined in 517 patients with non-Hodgkin's lymphoma treated at the National Cancer Institute. A significant excess of acute nonlymphocytic leukemia was found (9 observed, 0.08 expected, O/E = 112.5). The risk was greatest in patients treated with intense radiation (7 cases), although 4 of these patients subsequently received intense chemotherpay as well. This experience resembles that for Hodgkin's disease, except that the occurrence of leukemia following radiation alone or chemotherapy alone in this study is more prominent.

11. The safety and efficacy of photochemotherapy (oral methoxsalen and ultraviolet--A radiation) for psoriasis was evaluated in a clinical trial involving 1,125 patients and 15 hospitals. This population previously had shown a substantial risk of cutaneous carcinoma in patients so exposed. To determine the potentially adverse effects of photoactive medications (e.g., diuretics and tranquilizers) taken in conjunction with photochemotherapy PUVA, the incidence of phototoxic reactions was studied. A significant increased rate of severe, acture toxicity was not observed, and it was concluded that photoactive drugs are not a contraindication to photochemotherapy. Photochemotherapy was also determined to be more effective and less costly than previous therapies.

12. Among 6,000 women with cervical cancer and treated with radiation in Connecticut, 449 developed second primary cancers compared to 313 expected (O/E = 1.4). The excess incidence was attributable to cancers of the lung (64/12.6), bladder (26/8.9), kidney (14/4.9), uterine corpus (39/22.2) and rectum (30/18.3). The excess cancer of the bladder (9/3.2), kidney (9/1.7) and rectum (16/5.5) observed 15 or more years after radiotherapy was consistent with the long latent period of radiation-induced solid tumors. An excess of corpus cancer (15/6.5) and ovarian cancer (6/2.2) occurring 15 years post treatment is consistent with a radiation etiology, although metastatic lesions might have contributed somewhat to this excess. A deficit of breast cancer was observed, possibly due to radiation-induced menopause or to reproductive factors peculiar to this group of women (e.g., early first pregnancy) that may be protective.

13. A study of children irradiated for tinea capitis (scal ringworm) has indicated long-lasting scholastic and mental health effects in exposed children compared to children in nonexposed comparison groups.

14. In a study of 1,005 women treated with radioactive iodine and 2,141 women treated with surgery for hyperthyroidism at the Mayo clinic, no increased cancer mortality was observed in the 131-I treated women although an overall excess mortality was apparent. The pattern of mortality by age, treatment, year of treatment, and time after treatment suggest that the increased overall mortality was a consequence of women with a poor survival expectation for 131-I therapy, and not as a result of the radiation exposure. In addition to mortality there was no increased risk of cancer in the 131-I group compared with the surgery treated women (RR=1.0) There arose however, a signigicant risk of thyroid cancer in 131-I treated women (RR=9) and also for organs of high 131-I exposure (RR=1.8), although the numbers are relatively small and the risk was limited to the first 5 years after treatment and no dose-response relationship was observed. These results have prompted an extended follow-up of the former USPHS thyrotoxicosis study to further evaluate the risk of cancer following radioactive iodine exposures. In addition no increased risk of breast cancer was observed in any subgroup of women, those treated by thyroid replacement therapy, or radioactive iodine, or surgery.

15. A man with gynecomastia and a family history of diverse cancers developed adenocarcinoma of the breast 30 years following childhood thymic irradiation. His cultured skin fibroblasts displayed abnormal in vitro sensitivity to ionizing radiation indicating impaired ability to repair damaged DNA.

16. An analysis of data in the Connecticut Tumor Registry found the patterns of thyroid incidence in birth cohorts to coincide with the widespread use of radiation for benign head and neck conditions between 1920 and 1959. A decrease in rates was indicated for persons born in the 1960s when such irradiation was discouraged.

Significance to Biomedical Research and the Program of the Institute: Information about the carcinogenic effects of radiation is required in order to balance risk and benefits in medical, economic, and recreational activities. Epidemiologic data relating cancer incidence and mortality to radiation exposure, especially with good information on dose and timing of exposure, can influence theories of carcinogenesis and motivate experimental research.

The Radiation Studies program exists to investigate further,and clarify, the established causal link between cancer risk and exposure to ionizing radiation and certain types of non-ionizing radiation. An immediate practical need is for risk estimates on which to base regulatory and other decisions about the use of nuclear and radiological technology in industry and medicine, and with which to assess the value of exposure avoidance as a means of cancer prevention. A particularly cogent reason for a high level of epidemiological effort in studies of radiation carcinogenesis, however, is that the study of radiation carcinogenesis appears to be a particularly promising approach to understanding carcinogenesis in general. In many exposed populations, quantitative

description of exposure to affected tissues is straightforward, an advantage that does not obtain for most other carcinogens; furthermore, epidemiological studies can draw upon the background of a vast literature of experimental and theoretical radiobiology, including radiation carcinogenesis in experimental animals as well as studies at the cellular and subcellular levels. The importance of radiation studies for understanding carcinogenesis is illustrated by a series of breast cancer incidence studies carried out by the Radiation Studies Section and others. With remarkable consistency, these studies indicate that sensitivity to radiation carcinogenesis can be heavily dependent on developmental factors but relatively independent of factors responsible for international differences in population rates, that there may be wide variations among tissues with respect to the form of the dose-response curve and dependencies on radiation quality and fractionation of dose, and that, in contrast to the wave-like temporal distribution of leukemia risk following radiation exposure, the latency period for some sites of radiation-induced cancer may parallel the dependence of population rates on age at observation for risk.

The program seeks information on 1) the relative sensitivities of various tissues to radiation carcinogenesis, 2) the influences of host factors and other exposures on the carcinogenic action of radiation, 3) the distribution over time of the excess cancer risk following radiation exposure, and 4) the influence of various dimensions of exposure, including dose, radiation quality, and fractionation and protraction of dose. A particular goal, pursued in response to a widely felt public health and regulatory need, is to provide information about the risk of cancer following exposure to low doses of sparsely ionizing radiation such as gamma rays and x-rays.

The approaches taken in the Radiation Studies program are, first, to ensure that maximum benefit is derived from existing epidemiological resources for investigating cancer risk in exposed populations; second, to aggressively develop data resources from exposed populations that have not been studied previously, but which offer unusual potential for new information; and third, to encourage the development, at NCI and elsewhere, of epidemiological and statistical expertise in radiation carcinogenesis. The first of these approaches involves extending the follow-up of exposed populations already studied by project members and others (e.g., the Japanese A-bomb survivors, the Massachusetts series of former tuberculosis patients exposed as young women to multiple chest fluoroscopies, the large international clinical series of women given radiotherapy for cervical cancer, the two series of patients treated by 131-Iodine for hyperthyroidism and thyroid cancer, and the Israeli series of persons given radiation treatment for tinea capitis during childhood) and conducting special substudies to investigate particular hypotheses. A major effort, involving financial support for collaborative studies, extended visits by project members to the Radiation Effects Research Foundation (RERF) in Hiroshima, Japan, and by Japanese scientists to NCI, and the permanent stationing of a RSS member at RERF, is being made to increase participation by program members in studies of the Japanese A-Bomb survivors. Finally, authors of published studies relating risk from a particular cancer to radiation dose in different populations are encouraged to collaborate in

a reanalysis of the combined material, using identical methods and assumptions for all data sets, so that differences and similarities can be highlighted and inferences strengthened. This has been done for breast cancer and is currently being done for thyroid cancer.

Investigations of previously unstudied populations account for, by far, the greater part of the effort and money expended under the project. Of these, the case-control studies of adult leukemia among members of prepaid health plans, salivary gland tumor cases obtained from AFIP files, and thyroid cancer cases from the Connecticut Tumor Registry (and later from tumor registries in several different countries), and cohort studies of childhood cancer in relation to in utero exposure to x-ray pelvimetry among twins in Connecticut and Sweden, have only short-term implications for the future of the program, since it is unlikely that further useful information can be obtained once the studies have been completed. Other exposure cohorts, on the other hand, can be followed as long as there are cohort members remaining at risk of radiation-induced cancer. Thus,the future of the Radiation Studies program,is to a great extent, being determined by cohort studies currently under way or under development, of cancer patient populations treated by radiation (of which the population of cervical cancer patients identified from tumor registries in different countries is by far the largest), men and older women who received multiple fluoroscopy exposures during treatment for tuberculosis, and patient populations given radiation therapy for benign disease. Since much of the expense of epidemiological studies of new cohorts is in locating cohort members and gaining access to records, later follow-up studies of these cohorts should be easier and less expensive. The international cohort of 200,000 cervical cancer patients treated by radiation and/or surgery, as identified from records in 30 clinics and 20 cancer registies in over 20 countries, is expected to be a resource for future study comparable in value to the LSS sample of Japanese A-bomb survivors and the British series of patients given x-ray therapy for ankylosing spondylitis. Development of this resource, including dosimetry and current epidemiological and pathology studies, is the most extensive project in the program.

Studies of cancer risk in populations exposed to low doses of ionizing radiation avoid problems of extrapolation of risk from high to low doses, but tend to be of little value because impracticably large sample sizes are required for adequate statistical power. Studies of large populations exposed to intermediate doses, on the order of 10-50 rads, offer the possibilities of adequate power and straightforward extrapolation of risk to lower doses. The feasibility of such a study depends on special circumstances, such as the existence of a common membership in a professional organization, that facilitate information gathering. Studies of cancer incidence among some 140,000 registrants in the American Registry of Radiation Technologists, whose occupational exposures to radiation, accumulated over many years, are high enough for acceptable statistical power with respect to the detection of at least some carcinogenic effects, according to currently accepted risk estimates, will thus fill an important role in the program. Another approach to the low-dose risk estimation problem is to study populations exposed to high cumulative doses received

in small fractions. According to current radiobiological theory, the effect of such exposures should be proportional to cumulative dose, and only minimal assumptions are needed to extrapolate risk estimates from such data to very low-dose levels. Since cumulative doses are high, acceptable statistical power can be obtained with samples of only a few thousand. The population of patients exposed to multiple chest fluoroscopies during treatment for tuberculosis, therefore, will continue to be a major resource of the program.

Dosimetry is a crucial aspect of radiation studies, especially when non-uniform or partial-body exposures are involved. Dosimetry for current studies of medically-irradiated populations involves the collaboration of radiation physicists at the Bureau of Radiological Health, M. D. Anderson Memorial Hospital and Cancer Institute, the Harvard Joint Center for Radiation Therapy, and collaborating institutions in other countries, in the development of sophisticated dosimetric procedures using experimental measurements with anthropometric phantoms and a Monte Carlo simulation algorithm developed at Oak Ridge National Laboratory. The current program is, in effect, creating a greatly expanded capacity for epidemiological dosimetry which can be expected to facilitate future studies.

The epidemiology of radiation carcinogenesis is concerned with questions that go far beyond the identification of radiation as a cancer risk factor for certain tissues. In particular, estimates of cancer risk from low-dose exposures to low-LET radiation must eventually depend on epidemiological data obtained at higher dose levels, but the nature of that dependence probably will be derived from radiobiological considerations, including the results of studies of experimental carcinogenesis, microdosimetry, and radiation effects, like chromosomal abnormalities but unlike cancer, that can be easily studied at low doses. Therefore, the development of statistical and epidemiological expertise in the area of radiation carcinogenesis depends to a large extent on closer association between persons involved in epidemiological, experimental, and theoretical approaches to the problem. Another likely benefit of such association would be to encourage experimental investigations of questions arising from epidemiological studies, such as the relative sensitivities to radiation carcinogenesis of pre- and post-pubertal, and pre- and post-menopausal, breast tissue. For these reasons, increased collaborative research involving experimental radiobiologists at the National Laboratories of the Department of Energy and elsewhere, like the current study of chromosome aberration frequencies in three exposed populations also under study for cancer risk, and possible reanalyses by project members of experimental dose-response data, are planned. An interdisciplinary symposium on radiation carcinogenesis, sponsored by the Radiation Studies Section, is planned for the near future.

Proposed Course: The cohort studies of populations exposed to medical and occupational radiation will continue. The large studies of irradiated cervical cancer patients and atomic bomb survivors will receive major emphasis. Selected case-control studies will be initiated, as will additional cohort investigations. A more thorough discussion of specific studies is presented in the previous section entitled "Significance to Biomedical Research and the Program of the Institute."

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Contracts in Support of this Project

TEXAS UNIVERSITY OF, M.D. ANDERSON HOSPITAL (NO1-CP-01047)

Title: Studies of Iatrogenic Cancer and Radiation Dosimetry

Current Funding Level: $60,000

Man Years: 1.5

Objectives: To provide radiation dosimetry necessary to estimate organ doses received during radiotherapy for cervical cancer. These measurements are essential to the assessment of radiation risks for our International Radiation Study of Cervical Cancer.

Methods Employed: Physics measurements are being made for x-ray machines and intracavitary isotopes (radium). These include orthovoltage, betatron, megavoltage x-ray machnies, Van de Graaff machines, and cobalt-60 units in addition to radium and cesium intracavitary sources. Abstracted data from all participating radiotherapy centers are also being evaluated with regard to dosimetry.

Major Contributions: The contractor has rapidly and efficiently developed a measurement program to obtain organ specific doses following treatment for cervical cancer. Three detailed progress reports have been written. Calculations of active bone marrow dose and measurements have been performed and compared with the results from a Monte Carlo computer technique in a mathematically described anthropomorphic phantom. Visits to several radiotherapy centers have been made and questionnaires prepared for radiotherapists and medical physicists to determine treatment methods.

Proposed Course: To apply the developed dosimetry techniques to the other U.S. hospitals collaborating in the international study of cervical cancer and to apply the developed dosimetry to other groups of women similarly irradiated for metropathia and other conditions.

Date Contract Initiated: September 25, 1980.

WESTAT, INC. (NO1-CP-01011)

Title: Support Services for Radiation Studies

Current Funding Level: $413,437

Man Years: 8

Objectives: The objectives of this contract is to obtain technical (nonprofessional), managerial, and clerical support for epidemiologic

studies. The contractor functions in a supportive role carrying out specific tasks and does not engage in independent research.

Methods Employed: All phases of support services are being supplied, including: (1) preparing data collection forms; (2) preparing manuals for abstracting, coding, interviewing and tracing; (3) tracing individuals to determine their vital status; (4) obtaining their consent to be interviewed; (5) interviewing or sending mail questionnaires; (6) obtaining death certificates; (7) abstracting, keying, editing, updating, and coding of data; (8) occassionally transporting biological specimens; (9) assessing exposure information; and (10) creating and manipulating data files.

Major Contributions: The contractor has provided support services for the following studies: (1) international radiation study of cervical cancer clinical followup; (2) registry case-control studies for the cervical cancer study; (3) questionnaire preparation for the x-ray technologist study; (4) the thyroid case-control interview study in Connecticut; (5) the registry case-control study of endometrial cancer following hormonal therapy for breast cancer; (6) the Veteran Administration adjuvant drug study eval-uations; (7) the clinical trial evaluations of MeCCNU; (8) the telephone questionnaire and second mailing for the TB-fluoroscopy breast cancer study in Massachusetts; (9) the study of second cancers in Hodgkin's disease patients in Connecticut; (10) the study of lung cancer following irradiation for breast cancer; (11) the case-control study of second cancers following childhood cancer; (12) the study of second cancers following treatment for ovarian cancer; and (13) the preparation of death tapes acquired from various states.

Proposed Course: Continued expansion of support for ongoing studies and newly developed studies.

Date Contract Initiated: June 29, 1980

SYSTEMEDICS, INC. (NO1-CP-01043)

Title: Support Services for Radiation Studies

Current Funding Level: $233,464

Man Years: 4

Objectives; Same as WESTAT (NO1-CP-01011). Two contracts had been awarded for this project.

Methods Employed: Same as WESTAT (NO1-CP-01011).

Major Contributions: The contractor has provided support services for the following studies: (1) risk of head and neck cancers following

irradiation in childhood for enlarged tonsils; (2) TB-fluoroscopy study of males in Massachusetts; (3) data analysis for the case-control study of leukemia and lymphoma; (4) data analysis for the matched analysis for the cervical cancer study; (5) data analysis for the tinea capitis study of scalp irradiation; (6) data analysis for the study of cancer following radioactive iodine treatments for hyperthyroidism; (7) the risk of second cancers following radioactive iodine therapy for thyroid cancer; and (8) the study of valium use among breast cancer patients.

Proposed Course; Although the computer support has been adequate, the field support has been inadequate. Thus the contract will be terminated this year and recompeted (RFP No. NCI-CP-FS-11018-77).

Date Contract Initiated: September 31, 1980.

CONTRACTOR TO BE SELECTED (NO1-CP1-1007)

Title: Support Services for Case-Control Studies in California Tumor Registry

Estimated Funding Level: $40,000

Estimated Man-years: 1

Objectives: To abstract medical and radiation therapy information on 384 cervical cancer patients who developed second cancers and 768 controls. These data will be incorporated into the International Radiation Study of Cervical Cancer. California is the only registry that participated in the End Results Program and that is not currently participating. The California Tumor Registry would prefer to abstract their own data rather than allow our abstractor access to personal identifiers.

Methods to be Employed: Cases and controls have already been selected. The contractor only has to have the existing abstract form completed for the study. The data will be computerized and an edited data tape prepared for NCI.

Estimated Initiation Date: June, 1981

CONTRACTOR TO BE SELECTED (RFP No. NCI-CP-FS-11008-77)

Title: Cancer Following Tonsil Irradiation: Physical Examinations and Blood Studies

Estimated Funding Level: $90,000

Estimated Man-Years: 1½

Objectives: (1) To determine by physical examination whether there is any increase in thyroid nodules and head and neck cancer in persons irradiated for enlarged tonsils during childhood; (2) to determine whether there has been a radiation effect on the parathyroid as determined by blood tests of calcium levels; and (3) to evaluate the impact of intense screening on the detection of radiation-related thyroid tumors and the assessment of radiation risk.

Methods to be Employed: The Environmental Epidemiology Branch has been collaborating with clinicians at the Children's Hospital Medical Center and, in addition, will now be collaborating with the Brigham and Women's Hospital where the exposed and non-exposed study subjects will be examined. The Brigham Medical Group will provide the examination rooms, nurses, physicians, laboratories, freezers for blood studies, and all other necessary items for the conduct of the physical examinations and blood studies.

Estimated Initiation Date: June, 1981

CONTRACTOR TO BE SELECTED (RFP No.to be assigned)

Title: Support Services for a Study of Cancer Following I-131 Therapy for Hyperthyroidism

Estimated Funding Level: $300,000

Estimated Man-Years: 6

Objectives: (1) To identify survivors of a large USPHS study of hyperthyroid patients treated between 1946-1964; (2) to ascertain the current location and vital status of these patients; (3) to mail questionnaires requesting information on current health status and any malignancies; (4) to obtain death certificates for all decedents and hospital discharge summaries; (5) to abstract additional medical histories when necessary; and (6) to confirm all reported cancers.

Methods to be Employed; Same as WESTAT, Inc. (NO1-CP-01011)

Estimated Initiation Date: July, 1981.

DEPT. OF ENERGY (IAG No. to be assigned)

Title: Studies on Radiation-Induced Chromosome Damage in Humans

Estimated Funding Level: $73,000

Estimated Man-Years: 2

Objectives: (1) To calculate dose-response curves for frequency of chromosomal aberrations of various types; (2) within study populations, to analyze these curves for variation by dose and age at exposure, and to compare to similar curves obtained for cancer incidence; (3) to compare curves among study populations to assess the influence of different exposure modalities.

Methods to be Employed: An interagency agreement is proposed with the Department of Energy to perform the desired evaluations. Chromosomal aberrations will be determined and analyzed in 600 subjects selected from 3 populations exposed to diagnostic and therapeutic radiation during the period 1930-1970, and which are currently under study by the EEB for late health effects in relation to individual dosimetry. These populations are cervical cancer patients given radiotherapy, tuberculosis patients given multiple chest fluoroscopies, and persons irradiated for lymphoid hyperplasia during childhood. About fifty non-exposed persons from each of these populations will be selected as controls. Blood specimens, drawn at the hospital where these persons were treated, will be analyzed at the DOE supported cytogenic laboratory at the Oak Ridge Associated Universities.

Estimated Initiation Date: July, 1981.

CONTRACTOR TO BE SELECTED (RFP NO. NCI-CP-FS-11018-77)

Title: Support Services for Radiation and Related Studies

Estimated Funding Level: $291,975

Estimated Man-years: 4

Objectives: This contract is to replace NO1-CP-01043 which is being terminated. The objectives are the same as in WESTAT, Inc. (NO1-CP-01011).

Methods to be Employed: Same as WESTAT, Inc. (NO1-CP-01011).

Estimated Initiation Date: August, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04500-04 EEB

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Methodologic Studies of Epidemiology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	W. J. Biot	Head, Analytical Studies Section	EEB NCI
OTHER:	J. H. Lubin	Staff Fellow	EEB NCI
	L. W. Pickle	Senior Staff Fellow	EEB NCI
	C. E. Land	Health Statistician	EEB NCI
	J. D. Boice, Jr.	Epidemiologist	EEB NCI
	E. J. Martin	Cancer Expert	EEB NCI
	R. Spirtas	Statistician	EEB NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Environmental Epidemiology Branch

SECTION
Analytical Studies Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives of this project are to develop, adapt, expand, and evaluate methodological procedures useful in epidemiologic studies of cancer. Methods of design and analysis of epidemiologic studies were given particular emphasis, with attention focusing on the adaptation and evaluation of multivariate logistic models for analysis of case-control data. Investigation of general epidemiologic techniques for studying environmental cancer continued, including second printing of a text on biostatistical methods and review of methods to evaluate the magnitude of low-dose radiation risks.

PHS-6040
(Rev. 2-81)

Project Description

Objectives: To develop, adapt, expand, and evaluate methodological procedures useful in epidemiologic studies of cancer.

Methods Employed: Basic research is undertaken on statistical techniques which are useful in a variety of epidemiologic settings. Computational algorithms are developed as necessary, and the methods are applied to epidemiologic data generated and collected by investigators in the Environmental Epidemiology Branch and elsewhere.

Major Findings: Several Branch members contributed to the adaptation and development of statistical methods useful in epidemiologic studies. Wide use in the field required the second printing of a general text which features a library of programs for epidemiologic analysis using a programmable calculator.

Research continued on methods of analysis of case-control data. A new method based on a recursion formula was developed which allows rapid computation of exact conditional likelihood estimates of the relative risk in matched case-control studies regardless of sample size, greatly extending the applicability of this useful technique. The extent to which unconditional logistic analyses overestimate odds ratios from matched data sets was examined in a simulation study. The use of Miettinen's confounder score was shown to be at times inappropriate or else may lead to a conservative bias in the estimated relative risk.

Several studies were conducted on the statistical analysis of cohort data. One report presented an alternate method for analysis by the Cox proportional hazards failure time model. Reports were prepared proposing a simple variance approximation for the Mantel-Haenszel chi-square statistic and a conversion factor for obtaining standardized mortality ratios (SMRs) from proportionate mortality studies. Methodologic issues were also explored with respect to descriptive and correlational studies, especially using the county-by-county mortality resource.

Several methodologic issues related to the study of radiation-induced cancers were reported. Epidemiologic studies into the effects of low-dose radiation were reviewed, while the necessity of undertaking studies in high-dose rather than low-dose populations was stressed. Parametric families for dose-response curves were used to incorporate information on aspects of exposure other than dose, such as radiation quality and protraction and fractionation. Bayesian procedures were explored to incorporate uncertainties of assumptions and variability in experimental data in assessing human dose-response data. Stochastic models were adopted for the evaluation of sputum cytology screening programs among uranium miners.

Work has continued on the development of and accessibility to the Branch computer program library. For case-control data, SAS pre-programmed subroutines to estimate and test relative risks and to facilitate logistic

modelling were expanded, with one computer program for regression analyses in general n-m matched case-control studies developed.

A guide to sample size requirements for use in familial studies of cancer was prepared. Data from several Branch case-control interview studies were combined in order to assess the comparability of information from surrogate respondents. Sibs were best able to respond to questions about the subject family or about events that occurred early in life, while spouses were best able to describe events that occurred during adult life.

Significance to Biomedical Research and the Program of the Institute: Research in statistical methodology will help provide means for adequate analyses of the epidemiologic studies carried on by members of the Branch as well as by epidemiologists in other institutions.

Proposed Course: Methods development and adaptation will continue, with particular emphasis on techniques applicable to the Branch's analytical epidemiologic studies program.

Publications:

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04501-04 EEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Case-Control Studies of Selected Cancer Sites

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R.N. Hoover	Head, Environmental Studies Section	EEB NCI
Others:	J.F. Fraumeni, Jr.	Chief, EEB	EEB NCI
	W.A. Blattner	Clinical Epidemiologist	EEB NCI
	P.H. Strasser	Epidemiologist	EEB NCI
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COOPERATING UNITS (if any) Department of Health of the State of New Jersey; Biometry Branch, NCI, Division of Cancer Control & Rehabilitation; Medicine Branch, NCI; 28 Breast Cancer Detection & Demonstration Project Centers; 10 SEER Centers in Continental U.S.; University of Oxford and Birmingham Univ., England

LAB/BRANCH
Environmental Epidemiology Branch

SECTION
Environmental Studies Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
10	6.0	4.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to utilize the case-control interview methodology to do in-depth evaluations of specific cancer sites. By the nature of this method, the exposures evaluated include many of the exposure categories under which other Branch studies are listed; however, either because there are numerous etiologic hypotheses to be tested, or because a tumor deserves an in-depth evaluation in an attempt to generate hypotheses, these types of studies cover a number of different exposure categories, and are therefore considered as a separate program area.

PHS-6040
(Rev. 2-81)

Project Description

Objectives: (1) To identify tumor sites for which there are a number of unusual demographic laboratory or clinical associations indicating the necessity to evaluate a broad range of potential exposures. (2) To identify populations in which these in-depth case-control evaluations can be most efficiently carried out. (3) To design, conduct, and analyze these intensive case-control studies.

Studies and Methods Employed: During this year the project has included 16 studies using the case-control method: 2 of breast cancer, 3 of bladder cancer, 1 of non-Hodgkin's lymphoma, 1 of mycosis fungoides, 1 of testicular cancer, 1 of kidney cancer, 1 of ovarian cancer, 1 of childhood bladder cancer, 1 of intraocular melanoma, 1 of multiple myeloma, 1 of childhood cancer in general, 1 of abnormalities of the uterine cervix, and 1 of benign breast disease.

(1) Breast cancer patients (1,554) identified by the Breast Cancer Detection and Demonstration Project (BCDDP), women with benign breast disease (1,574), and normal screenees (1,391) were interviewed in their homes to collect information about risk factors for breast cancer and use of oral contraceptives, other exogenous estrogens, antihypertensive agents, thyroid medications, and major tranquilizers. Histological and clinical data were collected from BCDDP records. Analyses of these data are currently underway.

(2) A continuation of the breast cancer study noted above is being designed. Primary control of this study is being shifted from the Division of Resources, Centers, and Community Activities (DRCCA) to this Divison (DCCP). Breast cancer patients (1,000-2,000) identified by the BCDDP, equal numbers of women with benign breast disease, and women with no breast disease will be interviewed at home to collect information about a range of potential risk factors. Histological and clinical data will be collected from BCDDP records.

(3) All bladder cancer patients (4,000) who were diagnosed in 1978 in five states and five metropolitan areas were identified, and controls (7,000) were drawn from the general population of the 10 geographic areas. Subjects were interviewed in their homes to collect data about saccharin use, smoking habits, occupational history, sources of drinking water, hair dye use, coffee-drinking, and medical history. Histological data were collected from pathology reports. Analyses of these data are currently underway.

(4) All bladder cancer patients (150) who were diagnosed in 1979 in greater Atlanta, and controls (150) from the general population have been interviewed, following the protocol described in (3). The additional cases and controls will permit assessment of exposures stemming from work with textiles and dyes. Such analyses are currently underway.

(5) Male residents of 3 Georgia counties (56) who were diagnosed with kidney cancer from 1975 to 1978 were interviewed, following an

abbreviated version of the questionnaire described in (3). Exposures to be analyzed include occupational history, drinking water sources, use of tobacco, artificial sweeteners, coffee and pain relievers, and history of diabetes and urinary problems. Matched controls will be selected from the population-based controls interviewed during 1978 and 1979 as part of the bladder cancer study.

(6) Bladder cancer mortality rates are unusually high for both sexes in relatively rural areas of New Hampshire, Vermont, Maine and eastern New York state. Because of this all persons whose cause of death was bladder cancer (450) and who died in New Hampshire and Vermont during 1975-1979 and controls (900) will be identified. The next-of-kin will be interviewed in their home to collect data about occupation, environmental industrial pollution, source and treatment of drinking water, French Canadian ethnicity, tobacco consumption, diet, and other host and environmental factors.

(7) A case-control study of cutaneous T-cell lymphomas (CTCL) is under way in a series of 300 patients who are being treated for CTCL at the Skin and Cancer Hospital of Temple University in Philadelphia, Pennsylvania. The study has been designed to determine whether there is an association between CTCL and several variables possibly related to its etiology, many of which have in common exposures of the host to chronic antigenic stimulation. The influence of environmental agents as carcinogens will also be explored.

(8) Data from a study of non-Hodgkin's lymphoma patients treated at the NIH Clinical Center and their sibling controls are currently being analyzed. The study consists of complete information on 91 cases and 121 controls. Risks for NHL by radiation exposure, occupational exposure and past drug usage are now being evaluated.

(9) Testicular cancer patients treated at the NIH Clinical Center, Walter Reed Army Hospital, and Bethesda Naval Medical Center (325), and controls treated in those hospitals for other cancers (325), are now being interviewed in the hospital or by telephone to collect information about their occupational and environmental exposures, medical history with emphasis on genital tract abnormalities, family history, and lifestyle. To date 241 testicular cancer cases and 180 cancer controls have been interviewed. Mothers of subjects are also being interviewed by telephone, and their medical records abstracted to obtain data on subjects' prenatal and early childhood exposures to drugs, hormones, and radiation.

(10) Ovarian cancer patients diagnosed between 1978 and June, 1981 in 25 Washington, D.C. area hospitals (350), and women hospitalized for other conditions (350), are now being interviewed in their homes to collect information about medical, family, reproductive and menstrual histories, use of exogenous estrogens, contraception, occupation, and smoking. Microscopic slides are being reviewed and questionnaires are being mailed to subjects' physicians to collect additional data.

(11) A case-control study of intraocular malignant melanoma was initiated in collaboration with Wills Eye Hospital in Philadelphia. The data

collection for the study has been completed. A total of 1,465 medical records were abstracted and 1,285 telephone interviews were completed. Analysis is under way.

(12) Multiple myeloma patients (180) newly diagnosed by the Acute Leukemia Group B and their siblings are being mailed questionnaires that collect data about occupation, therapeutic drugs, medical and family history, and chronic antigenic exposures.

(13) The Environmental Epidemiology Branch is conducting a case-control study of childhood bladder cancer in cooperation with investigators participating in the SEER Program. The study has been designed to determine whether childhood bladder cancer is associated with pre- or post-natal exposures to known or suspected bladder carcinogens such as artificial sweeteners and cigarette smoking. Physicians of cases and controls have been contacted for permission to interview mothers.

(14) A study of the relationship between the occurrence of chicken pox during pregnancy and subsequent cancer in the offspring was conducted using the Oxford survey of childhood cancer.

(15) To identify risk factors for various cervical abnormalities, 237 women with abnormal cervical smears (65 carcinoma in situ, 81 severe dysplasia, 44 mild dysplasia and 47 normal histology) and 422 control women were interviewed and the data analyzed in collaboration with investigators at Oxford University.

(16) A study of benign breast diseases was conducted, also in conjunction with epidemiologists at Oxford University, in order to assess similarities in patterns of risk for benign breast disease and breast cancer.

Major Findings: (1) The National Bladder Cancer study has been under analysis in the past year. The issues being addressed include further evaluations of risks associated with artificial sweetener use, coffee drinking, hair dye use, work in the chemical and leather industries, cigarette smoking, drinking water quality, urinary tract infection, and an in-depth analysis of the possible occupational explanations for the high bladder cancer rates in Detroit. Among the preliminary findings are no obvious association of risk with use of hair dyes, a diminished risk associated with never having drunk coffee (but no dose-response relationship among coffee drinkers), and a two-fold risk associated with multiple urinary tract infections.

(2) Findings from a study of benign breast disease conducted in Oxford, England revealed that few of the risk factors previously identified for breast cancer related to the occurrence of benign disease. The factors evaluated included age at first livebirth and number of livebirths. However, the study did find that obese women and those of the higher social classes were at an excess risk of developing benign lesions; these findings may reflect diagnostic biases. In addition, this study allowed

evaluation of the relationship of oral contraceptive usage to risk of various benign abnormalities. An inverse association was found between use of oral contraceptives and risk of fibroadenoma, histologically confirmed chronic cystic disease, and breast lumps not subjected to biopsy. Current users of the pill had the lowest risk, particularly when use was for an extended period. In contrast, past users demonstrated no reduction in risk. The reduction in risk for chronic disease appeared predominately for users of high progestogen-containing pills.

(3) Factors associated with risk of mild cervical dysplasia, severe dysplasia, and carcinoma in situ were similar to those previously identified for invasive carcinoma and included early age at first intercourse, multiple sexual partners, and pregnancy outside marriage. Analysis to disentangle correlated factors revealed that number of sexual partners exerted effects independent of age at first intercourse, whereas the reverse was not true. The findings fails to support suggestions that adolescence is a period when the cervix is most vulnerable to the effects of sexual behaviour. Other risk factors identified included smoking and use of oral contraceptives.

Significance to Biomedical Research and the Program of the Institute: The case-control methodology provides a rapid, relatively inexpensive yet scientifically rapid way of assessing the relationship between a disease and a wide variety of potential causes of that disease (occupational, general environmental, life-style, genetic, etc.). This method is the usual one first employed by epidemiologists to test hypotheses that have come from clinical observations, laboratory experiments, or descriptive epidemiologic efforts. Because of the speed with which these studies can be performed and the wide variety of potential causes that can be assessed simultaneously, these studies often provide the first sound scientific evidence of a preventable cause of malignancy; evidence which can then be acted upon through educational programs or regulatory actions. As such, this type of work is a key element in identifying preventable causes of malignancy in humans.

Proposed Course: (1) The studies of multiple myeloma, mycosis fungoides, bladder cancer in children, and the second study of breast cancer in the BCDDP, will continue in the phase of data collection during the next year.

(2) The field phase of the studies of testicular cancer, ovarian cancer and bladder cancer in New England will be completed and analyses will begin.

(3) The first breast cancer study in the Breast Cancer Detection Demonstration Project, the bladder and kidney cancer studies in Atlanta, the non-Hodgkin's lymphoma study, and the intraocular melanoma study have all completed data collection and will continue to be analyzed during the next year.

(4) Continuous evaluations will be made of numerous tumor sites in order

to identify those tumors for which intensive case-control studies would be the most appropriate next step in evaluating potential etiologic hypotheses. Avenues for appropriately achieving these case-control evaluations will be explored. Steps are currently being taken to implement such evaluations of invasive cancer of the uterine cervix.

(5) The National Bladder Cancer Study will continue under analysis to assess exposures other than artificial sweeteners during the next year.

Publications:

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04779-05 EEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Field Studies in HIgh-Risk Areas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)
Center for Disease Control; Environmental Protection Agency, Virginia Health Department; University of North Carolina; University of Texas; University of Minnesota; Louisiana State University; University of Miami

LAB/BRANCH
Environmental Epidemiology Branch

SECTION
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INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
7.5	7.0	.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives of this project are to identify and describe environmental and host determinants of cancer in areas of the U.S. at high risk of cancer through the use of analytical epidemiologic techniques, particularly case-control studies of specific cancers. Completed during the year were interview studies of (a) lung cancer in Jacksonville, Florida which found that increased risks associated with employment in the shipbuilding, construction, and lumber forestry industries contributed to this area being ranked first among all urban counties in the country in lung cancer mortality among white males; (b) oral cancer among women in North Carolina, showing that chronic oral snuff use is responsible for the high rates of this tumor in the southeast; and (c) esophageal cancer among black men in Washington, D.C. revealing increased rates with heavy alcohol consumption and with decreased nutritional status. Field investigations were also underway of renal cancer in Minneapolis; lung, pancreas and stomach cancer in Louisiana; respiratory cancer in Texas; nasal cancer in Virginia and North Carolina; bladder cancer in rural New England; and breast cancer in Alberta, Canada.

PHS-6040
(Rev. 2-81)

Project Description

Objectives: To identify and describe the environmental determinants of cancer in areas of the United States where cancer rates are high.

Methods Employed: Field studies are conducted in areas of the country where cancer rates are high and etiologic hypotheses can be tested. The studies are generally case-control investigations whereby cancer patients and controls, or their next-of-kin in the event they had died, are interviewed regarding lifetime histories of residence, occupation, tobacco consumption, diet, and medical or other factors. Comparison of responses between the cases and controls are then made by analytical epidemiologic techniques to identify, estimate, and evaluate cancer risk factors. When a particular suspect environmental or occupational exposure among a well-defined population group is recognized, cohort investigations may be initiated to determine the group's cancer experience. Often both the case-control interview and the cohort studies are preceded by reviews of appropriate death certificates and medical records for cancer cases and controls for comparisons of available information.

Major Findings: A series of case-control investigations are ongoing in areas of the U.S. where mortality rates for particular tumors are high. A major effort continued to evaluate risk factors for lung cancer, the leading cause of cancer death among men in the United States. Previous Branch investigations of lung cancer in coastal areas of Georgia, and lung cancer and mesothelioma in Tidewater, Virginia found significantly increased risks associated with employment in the shipbuilding industry, particularly during World War II. This year a study involving 321 lung cancer patients and 431 controls was completed in Duval county (Jacksonville) Florida, which ranked first in lung cancer mortality 1970-75 for white males among all urban counties in the country. Shipyard employment was a contributor to the excess, with over 20% of the cases having worked in the industry, most for limited periods during the war. Asbestos is suspect and seems involved in the increase found among construction workers as well. Excesses of from 50% to 3-fold were also linked to the lumber, forestry, and fishing industries, but occupational factors could not fully account for the areas' exceptionally high lung cancer rates. Migration, smoking, and diagnostic patterns seemed unrelated to the clustering of high mortality, raising the possibility of a role for general environmental factors such as sulfate air pollution, high humidity, or radon exposure in homes and buildings.

A small case-control survey of lung cancer in Bath, Maine, site of the oldest shipbuilding company in the United States, brought to 4 the number of Branch interview investigations showing an increased risk of lung cancer associated with employment in U.S. shipyards. Combining data from the more than 2500 interviews placed the relative risk, adjusted for smoking, for employment in the industry in the 1940's at about 1.4, suggesting that as many as 100,000 extra lung cancer deaths may eventually result among the cohort of some 4.5 million Americans who worked in ship construction and repair during World War II.

A study of respiratory cancer was also begun in collaboration with the University of Texas School of Public Health to evaluate the high risk of respiratory cancer among both sexes in coastal Texas, and interviewing continued to obtain detailed information on characteristics of cancer patients in Louisiana, as part of a collaborative (with the EPA and Louisiana State University) case-control interview study in Louisiana for lung, pancreatic, and stomach cancers. Lung and bladder tumors were also studied in a broad-based epidemiologic study in New Jersey in collaboration with the State Department of Health.

A correlation study previously published by the Branch revealed that nasal cancer mortality was high in counties with furniture manufacturing industries. Subsequent examinations of death certificates from North Carolina, where the industry is most heavily concentrated, showed a 4-fold excess of this tumor associated with individuals for whom furniture manufacturing was listed on the certificate as the usual occupation. Although nasal cancer is rare and the number of cases small and spread over a fairly wide geographic area, interviews (by telephone) of cases diagnosed in Virginia and North Carolina over the past 10 years were conducted to further quantify risk factors for this cancer.

Esophageal cancer is relatively rare among whites, yet this tumor accounts for more deaths than any, except lung and prostate cancers, among black men in Washington, D.C. To attempt to uncover reasons for this excess, next-of-kin of black males who died of esophageal cancer and other causes (controls) since 1975 were interviewed for information regarding the deceased's residence, occupation, tobacco and alcohol consumption, diet, and other factors. Analysis conducted during the year showed alcohol consumption to be the strongest risk factor, with the risk rising to about eight-fold among heavy consumers (one pint or more per day). Cigarette smoking, after controlling for alcohol consumption, was linked to esophageal cancer in this series, but the association was much weaker (relative risk = 1.5). One of the more interesting findings related to nutrition, with decreased intake of fruits and vegetables, fresh meats, and dairy products and eggs among the cases.

Alcohol, combined with cigarette smoking, was also incriminated as a risk factor for cancer of the mouth and throat in a case-control interview study among southern women completed during the year in collaboration with the University of North Carolina. Snuff use, however, was identified as the cause of the sharply elevated mortality from oral cancer among females in the South. Forty percent of the cases were snuff dippers. The increase associated with the habit was 4-fold for non-smokers: for cancers of the cheek and gum, where the tobacco powder is usually placed, the risk reached nearly 50-fold among long-term users.

Renal cancer mortality and incidence rates are high in the north central part of the U.S. A large (approximately 590 cases and 1180 controls) interview study was conducted during the year in collaboration with the University of Minnesota. The response rate was remarkable, interviews being completed with over 97% of those contacted. Analyses of the collected data are focusing on diet and beverage intake, smoking, occupation, and ethnic background among other charcteristics. In addition, peripheral

leukocytes from patients under age 45, patients with bilateral tumors, and patients with a family history of renal cancer are being assayed for specific chromosomal abnormalities which may serve as markers for this neoplasm.

Analyses of interview data from a small case-control study of colorectal cancer in rural Nebraska, where rates have been high in the past, confirmed the hypothesis from an earlier Branch correlation study that risk was elevated among persons of Czechoslovakian descent. The association seemed due in part to dietary factors, particularly with diets high in fats, sweets, and refined foods.

Staff members of the Branch are collaborating with the W. W. Cross Cancer Institute in a large case-control study of breast cancer in Edmonton, Canada, in the province of Alberta, where rates for breast cancer are among the highest in the world. The study is unique in that nearly all cases throughout the province are interviewed by the Institute. With the cooperation of the Alberta Health Care Insurance Commission, population-based controls were identified and nearly 1000 interviews among adult female residents conducted. Reported during the year were contrasts of differences in risk factors according to age when breast cancer was diagnosed. Among women over age 45, late age at first birth, low parity, late age at menopause, and not having breast fed were linked to increased risk. Among women below this age, late age at menarche and recent use of birth control pills were identified as risk factors. Late age at first birth, difficulty in conceiving, previous benign breast disease, and a history of breast cancer among mothers and sisters were associated with higher risk at all ages.

Significance to Biomedical Research and the Program of the Institute: These studies allow the testing of hypotheses regarding the etiology of cancer in the United States. Answers obtained may lead to the recognition of cancer hazards and may directly suggest actions that need be taken to prevent the exceptional rates of cancer occurrence in the high-risk areas of the country.

Proposed Course: Field studies in areas where cancer rates are high will continue. Analysis of the information collected in Pennsylvania, North Carolina, Virginia, Minnesota and Alberta will be completed in the coming year, with interviews continuing in Louisiana, Texas and rural New England. Results from these ongoing studies will help suggest where further epidemiologic research will be worthwhile.

A new study of colorectal cancer will be started in retirement areas of Florida, focusing on identifying reasons for the unusually low risk of this cancer among males and females. This epidemiologic investigation will also include a laboratory component to assess several nutritional and metabolic indices which may play a role in colon carcinogenesis. Case-control studies of rare cancers in several urban areas of the country are also planned. Also planned is an interview study of esophageal cancer among blacks in southeast Atlantic coastal areas, particularly

Charleston, South Carolina, where rates have been historically high. Under consideration are epidemiologic studies in collaboration with the Cancer Institute of the Peoples' Republic of China to identify causes for the exceptional incidence of esophageal tumors in certain rural areas, including the initiation of a population-based nutrition intervention trial to evaluate the potential protective effects of carotene, ascorbic acid, and riboflavin supplements.

Collaborative studies with other governmental agencies (EPA, CDC, NIOSH, FDA, NCHS) will continue to be expanded so that a coordinated approach to field studies of cancer in the U.S. will be established with each agency contributing according to its strengths.

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Mason, T.J. and Pickle, L.W.: Etiologic Study of Respiratory Cancer in Coastal Texas. In Proceedings of the First NCI/EPA/NIOSH Collaborative Workshop: Progress on Joint Environmental and Occupational Cancer Studies, Rockville, MD., May 6-8, 1980. Washington, D.C., U.S. Government Print. Off., 1981, pp. 88-93.

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Ziegler, R.G., Morris, L.E., Blot, W.J., Pottern, L.M., Hoover, R.N., and Fraumeni, J.F., Jr.: Esophageal cancer among black men in Washington, D.C. II. The role of nutrition. JNCI (In press)

CONTRACTS IN SUPPORT OF THIS PROJECT:

WESTAT, INC. (NCI-CP-01044)

Title: Support services for epidemiologic studies

Current Funding Level: $1,431,424

Man Years: 45

Objective: To provide technical, managerial, and computer support for epidemiologic studies of cancer, including those in high risk areas.

Methods: A management system has been established to facilitate direct communication between NCI scientific investigators and the Westat program director, project managers, computer specialists, and field supervisors so that all phases (design, forms preparation, conduct, quality control, and analysis) of field studies can progress efficiently.

Major Contributions: Field studies were completed for oral cancer in North Carolina, esophageal cancer in Washington, D.C., breast cancer in Alberta, Canada, and lung cancer in Virginia and Florida. Interviewing and/or computational support were conducted for studies of renal cancer in Minnesota, bladder cancer in New England, Louisiana and Georgia, nasal cancer in North Carolina and Virginia, eye cancer in Pennsylvania, and lung cancer in Maine. Planning support was provided for a study of colorectal cancer in Florida.

Proposed Course: Support for a variety of epidemiologic investigations will continue as needed to enable the Branch to answer critical questions about the environmental and host determinants of cancer.

LEHIGH UNIVERSITY (NO1-CP-81038)

Title: Support services for epidemiologic studies of lung cancer in communities with nonferrous smelters

Current Funding Level: $77,233

Man Years: 2.8

Objectives: To provide interviewing, data preparation, and environmental measurement support services for a case-control study of lung cancer in eastern Pennsylvania.

Methods: An interviewing and medical records abstract team was established to carry out the field data collection phase of this case-control study and assemble information on area environmental pollutants.

Proposed Course: This contract was terminated upon completion of the support services.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 05128-02 EEB

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Nutritional Factors in Cancer Etiology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	W.J. Blot	Head, Analytical Studies Section	EEB NCI
	L.W. Pickle	Staff Fellow	EEB NCI
	J.F. Fraumeni, Jr.	Chief, Environmental Epidemiology Branch	EEB NCI
	E.J. Martin	Cancer Expert	EEB NCI
	D.M. Winn	Staff Fellow	EEB NCI
	J.H. Lubin	Staff Fellow	EEB NCI
	L.E. Morris	Epidemiologist	EEB NCI
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COOPERATING UNITS (if any)
National Center for Health Statistics
National Institute on Aging
Cross Cancer Institute, Alberta, Canada
New Jersey Department of Health
Louisiana State University

LAB/BRANCH
Environmental Epidemiology Branch

SECTION
Environmental Studies Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.3	0.2

CHECK APPROPRIATE BOX(ES)

[X] (a) HUMAN SUBJECTS [ ] (b) HUMAN TISSUES [ ] (c) NEITHER

[ ] (a1) MINORS [X] (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Dietary exposures being assessed in human populations include consumption of specific food groups, such as meat or fruits and vegetables or the Brassica genus; and specific foods, such as beef or ethnic items; macronutrient and micronutrient intake, such as fat, vitamin A, carotene, or vitamin C; general nutritional status; metabolic indices, such as serum cholesterol or serum vitamin A; food additives; and cooking practices. Cancers being studied include those of the colon, rectum, breast, esophagus, lung, pharynx, and oral cavity, pancreas, stomach, and kidney. Case-control studies have been initiated in high-risk areas with unusually high mortality from cancers conceivably related to diet and among migrants whose changing cancer rates appear related to new lifestyles such as Japanese-Americans. Case-control studies have also been designed to test specific hypotheses generated by animal or in vitro experiments. Data from HANES I and II and the USDA Food Consumption Survey are being analyzed to test specific hypotheses, such as the relationship of age at menarche to diet, and to provide descriptive information on U.S. dietary patterns, diet variation, and nutrient intake and its determinants.

Project Description

Objectives: 1) To assess in human populations specific hypotheses concerning the relationship of diet and cancer that have been suggested by biochemical, animal, clinical, and epidemiological studies. Such hypotheses may concern specific food items, food groups, macro or micronutrients, food additives, cooking and processing practices, biochemical measures related to diet, anthropometric parameters related to diet, general nutritional status, etc. Cancer may be initiated, promoted, or inhibited by such exposures.

2) To search for relationships between diet and specific cancers in those especially high or low risk areas identified by the U.S. cancer maps where diet could conceivably be involved in generating the observed geographical patterns.

3) To develop and utilize national nutrition data resources that might contribute to cancer epidemiology. One example is HANES I, the first Health and Nutrition Examination Study of the American people, conducted on a national sample of 23,000 people.

4) To develop methods for nutritional epidemiology, including diet-related questionnaires, logistics for appropriate laboratory tests, and analytic approachs.

5) To elucidate the basic biology of carcinogenesis through studying in human populations the influence of diet on cancer, whether directly or via the endocrine or immune systems.

Individual Studies: Rationale, Methods, and Status

Studies numbered 1,2,3,5,10,11, and 12 represent the nutrition-related components of cancer epidemiology studies described in more detail in Project Z01-CP-04779-05-EEB, Field Studies in High Risk Areas.

1) A death certificate-based, case-control study of esophageal cancer was initiated among black male residents of Washington, D.C., the U.S. metropolitan area with the highest rate of esophageal cancer among black males. Next-of-kin of 120 black men who died of esophageal cancer in 1975-77 and of 250 black men of similar age who died in 1975-77 of other causes were interviewed. Information was collected on dietary patterns (usual adult frequency of consumption of 31 food items, prior to 1974), unusual substances eaten, cooking practices, alcohol consumption, and other relevant exposures. Two papers on this research have been accepted for publication.

2) A case-control study of colorectal cancer was conducted in two rural farming counties in eastern Nebraska with unexpectedly high rates of colon cancer. Many people of Czechoslovakian ancestry live in this area; and Czechoslovakians, when compared to other immigrants to the U.S., show elevated rates of colon cancer. Since their traditional ethnic diet might be involved, emphasis was placed on assessing diet in this study. Interviews were completed for 58 colon cancer cases

and 28 rectal cancer cases, all diagnosed during 1970-77, and for 176 hospital controls of similar age, sex, county of residence, and place and year of hospitalization. Next-of-kin constituted 56% of the interviews. Information was collected on dietary patterns (usual adult frequency of consumption of 57 food items, including ethnic foods), cooking and preserving practices, wine and beer consumption, and other relevant exposures. The data are now being analyzed.

3) A population-based case-control study of breast cancer was conducted in Alberta, Canada in collaboration with Cross Cancer Institute, Edmonton. Breast cancer rates in Alberta are among the highest in the world. Interviews were conducted with 571 breast cancer cases diagnosed during 1975-77 and with 826 population controls. Questions were asked about the usual adult frequency of consumption of 8 food items. The data are now being analyzed.

4) A population-based case-control study of colorectal cancer is being initiated in the three regions of Florida with high rates of immigration from the Northeast and North Central states. The U.S. cancer maps showed that colorectal cancer mortality rates for white men and women were lower in the South by about 50%, than in the Northeast or North Central states. This regional gradient in risk could not be explained by differences in income or population density between North and South. Close examination of the age-specific cancer mortality rates for those counties in Florida where many Northerners move at retirement revealed that in these counties colorectal cancer rates were as low as in Southern counties of comparable population and did not rise toward the Northern rates at the older retirement ages.

This preliminary study will define the characteristics of this apparent reduction in colorectal cancer risk on migration, quantify it, and see whether it might be due to some change in lifestyle, possibly diet or drinking water, or to the migrants being a self-selected subset of Northerners. The case series, selected from the 1979 Florida mortality tape, consists of 1,160 white colon cancer deaths and 205 white rectal cancer deaths, 25-79 years of age, whose usual place of residence was one of the eleven Florida counties with heavy immigration. The two control series, also drawn from the 1979 Florida mortality tape, consist of 1,021 controls who died of a cancer other than colon, rectum, or breast and 700 controls who died of a cause other than cancer. Both control series were frequency-matched to the case series on age, sex, and usual county of residence. Questions will focus on residential history; medical history; social, economic, and demographic characteristics; and a few indicators of general dietary patterns (usual adult frequency of consumption of 11 food items, before and after migration to Florida). The data are now being collected.

5) A population-based case-control study of lung cancer, with a dietary component, was initiated in collaboration with the New Jersey State Department of Health in those areas of New Jersey showing unusually high lung cancer mortality rates on the U.S. cancer maps. Since many animal and cell culture studies have indicated that pharmacologic

doses of retinoids can protect against development of cancer, vitamin A has been postulated to reduce cancer risk. The few relevant epidemiological studies of dietary patterns and cancer have suggested a broader association: fruit and vegetable intake in general was elevated among those at less risk. The dietary component of this study was designed to assess whether vitamin A activity, retinol, carotene, vitamin C, all fruits and vegetables, or a subclass of fruits and vegetables, such as the Brassica genus, is associated with reduced risk of lung cancer. The study will also evaluate the interaction between smoking, occupational exposure, and pollution, and diet, in order to evaluate the potential of diet to alter risk at a later stage of tumorigenesis. Usual adult frequency of consumption, prior to 1975, of 44 food items and a history of vitamin pill usage are being collected. Approximately 700 lung cancer cases diagnosed in 1980-81 and 700 population controls of comparable age, sex, race, and residence will be selected; an estimated 40% of the interviews will be with next-of-kin. Interviewing, which is being handled by the New Jersey State Department of Health, will continue through 1981.

6) HANES I, the Health and Nutrition Examination Survey conducted in 1971-74 by the National Center for Health Statistics, collected dietary, biochemical, clinical, and anthropometric information on the nutritional status of a national sample of the U.S. population comprising 23,000 persons. With data from HANES I, regional differences in vitamin A and vitamin C intake and related dietary patterns are being assessed to see whether such differences could explain the North-South gradient in colon, rectal, and breast cancer mortality noted in the U.S. cancer maps. For these three cancers mortality rates were higher in Northeast and North Central states than in the South, and the difference could not be explained by socioeconomic status or population density. Intake of vitamin A, retinol, carotene, and vitamin C, based on 24-hour recalls; intake of vitamins A and C based on food frequencies; frequency of fruit and vegetable consumption; and serum vitamin A levels are being compared, after adjustment for sex, race, and age, with analysis of variance and regression techniques.

7) With the serum vitamin A data collected in HANES I on a national sample of 13,000 adults, possible determinants of serum vitamin A levels are being evaluated: sex, race, age, poverty status, pregnancy-lactation status, region, diet, and individual variation. Regression and analysis of variance techniques are being used. Since several prospective studies have recently shown that mean serum vitamin levels were lower prior to disease, among those that eventually developed cancer than among controls, the determinants of serum vitamin A levels are of interest. A specific hypothesis being tested is whether within a population as well-fed as that in the U.S., serum vitamin A levels are not significantly affected by vitamin A intake since intake is generally more than adequate.

8) With the HANES I dietary and anthropometric data collected for approximately 100 women between the ages of 12 and 18, various food groups, macronutrients, and body measurements are being evaluated as

predictors of age at menarche. In international comparisons, late maturation and age at menarche are inversely correlated with risk of breast cancer and may be indicators of the dietary patterns that promote this disease.

9) In 1981-82 the Environmental Epidemiology Branch, in cooperation with the National Institute on Aging, several other Institutes, and the National Center for Health Statistics, will trace and re-interview, if still living, 14,000 adults examined in HANES I 5 to 10 years earlier. By collecting intervening cancer morbidity and mortality for this cohort, associations between dietary patterns prior to disease and the common cancers can be determined. Once these people are traced in 1981-82, further cancer mortality will be followed with the National Death Index.

10) A case-control study of oral and pharyngeal cancer was conducted among women in central North Carolina since the U.S. cancer maps revealed excess mortality from these two cancers among white women in the Southeast U.S. Interviews were completed for 232 cases, who were diagnosed or died in 1975-78, and 410 controls individually matched on age, race, county of residence, and source of ascertainment. Next-of-kin provided 60% of the interviews. Information was collected on dietary patterns (usual adult frequency of consumption of 22 food items), unusual foods eaten, methods of cooking, and alcohol consumption. The data are now being analyzed.

11) A case-control study of lung, pancreas, and stomach cancer was initiated in southern Louisiana in collaboration with Louisiana State University because of the relatively high mortality rates for these three cancers in this region. The study sample will consist of approximately 1,200 lung , 200 stomach, and 175 pancreatic cancer patients and an equal number of hospital controls, individually matched by age, sex, race, parish of residence, and hospital. Next-of-kin will provide approximately 15% of the interviews. Information is being collected on dietary patterns (usual adult frequency of consumption of 57 food items prior to disease), food preparation and storage practices, beverages consumed, spices used, source of drinking water, and alcohol consumption. The study will be in the field through 1981.

12) A population-based case-control study of kidney cancer was conducted in Minneapolis because of the extremely high kidney cancer mortality rates in this city. Approximately 600 cases and 1,200 controls were selected; 33% of the interviews involved next-of-kin. Information collected includes dietary patterns (usual adult frequency of consumption of 30 food items, five years earlier), cooking practices, alcohol consumption, and an extensive beverage and drinking water history. The data are now being analyzed.

Major Findings: 1) In the study of esophageal cancer among Washington D.C. black males, five general measures of nutritional status -- fresh or frozen meat and fish consumption, dairy product and egg consumption, fruit and vegetable consumption, relative weight (weight/height^2), and

number of meals eaten per day -- were each statistically significantly and negatively correlated with the relative risk of esophageal cancer. The least nourished third of the study population, defined by any of these measures, was at twice the risk of the most nourished third. When the three food consumption measures were combined into a single overall index of dietary patterns, the relative risk of esophageal cancer between extremes was 14. The association of each nutrition measure with esophageal cancer was not markedly reduced by controlling for ethanol consumption, smoking, socioeconomic status, or the other nutrition measures. Estimates of vitamin A, carotene, vitamin C, thiamin, and riboflavin intake were inversely associated with relative risks; but each micronutrient index was less strongly associated with risk than the broad food groups that provide most of the micronutrients. Thus, no specific micronutrient deficiency was identified. Instead, generally poor nutrition was the major dietary predictor of risk and may partially explain the susceptibility of urban black men to esophageal cancer.

2) In the study of colorectal cancer in rural Nebraska, the excess risk was primarily among the people of Czechoslovakian ancestry. This elevated risk seems related to Czech life-style, rather than to specific high-risk families among the Czechs since Czech ancestry was not related to excessive familial clustering of cancer at any site. No specific foods or food groups could be identified as risk factors for colon and rectal cancer for the entire population in the study. However, risk was elevated among those Bohemians who consumed high levels of meat, dairy products, grains, and sweets. Whether other Americans are similarly affected by a high fat diet needs to be further investigated.

3) In the study of breast cancer in Alberta, Canada, relative risk increased with increasing frequency of consumption of beef, pork, cheese, and desserts and decreased with frequency of fish and poultry consumption. Of the food items, food groups, and macronutrients whose intake could be estimated with the very limited data, breast cancer risk was the most strongly associated with red meat consumption, reaching a relative risk of about 2 when the upper and lower quantiles of intake were compared. The association with red meat consumption was not markedly reduced by adjusting for age at menarche, age at natural menopause, or relative weight (W/H^2), which are indirect indicators of an affluent Western diet, nor by adjusting for the other known risk factors for breast cancer. The associations noted between diet and breast cancer held whether the disease had been diagnosed before or after menopause.

Significance to Biomedical Research and the Program of the Institute:

General dietary patterns, nutritional status, specific foods and food groups, and food additives are being recognized as possible causes of cancer. The American people seek guidance on diets to minimize their risk of cancer, and Congress and the Executive Branch must determine what should be regulated. Epidemiological studies of diet and cancer can contribute to a rational basis for public policy

decisions. It is necessary to test and quantify in human populations those hypotheses about the role of diet in carcinogenesis that have resulted from animal studies, in vitro experiments, and clinical observations. Nutritional epidemiology can also reveal broad correlations between dietary patterns and cancer, which then serve as the basis for further laboratory research, and further analytic epidemiology.

Certain diets and foods seem able to initiate carcinogenesis; others seem able to promote it; while still others seem able to reduce cancer risk. Their mechanism of action can be direct through interaction with DNA or indirect through alteration of metabolic pathways or cell regulation or even more indirect through modification of the endocrine or immune systems. Further research on diet and cancer, in which both epidemiology and laboratory science must cooperate, could yield insights into these mechanisms and the biology of carcinogenesis.

Proposed Course: For all the individual studies mentioned, analysis and publication of results will follow the field work. Assessment of specific hypotheses concerning the relationship of diet and cancer, and generation of hypotheses by exploratory case-control studies in high risk areas, will continue.

If the preliminary death certificate-based study of colorectal cancer among Florida migrants indicates that the apparent reduction in risk among Northerners moving to Florida is not attributable to their being a self-selected subset, then a more comprehensive case-control study of incident colorectal cancer cases, and possibly breast cancer cases also, will be initiated in Florida retirement areas. This study will help to identify the attributes of the Southern environment or lifestyle that may be involved in reducing cancer risk -- possibly increased consumption of fruits and vegetables, or more vitamin A or C, or the quality of the drinking water.

Research utilizing HANES I data will continue; and the USDA National Food Consumption Survey, which consists of 3 consecutive 24-hour recalls by a national sample of 69,000 people, will be added to the Branch's data resources. In addition to being utilized to test specific hypotheses related to diet and cancer, these data sets will also provide descriptive information necessary for the rational design and analysis of nutritional epidemiology studies. For example, they can be used to identify the foods that are the major sources in the U.S. diet of various micronutrients; the foods that are generally eaten by Americans of different ages, races, and regions; and any patterns inherent in the U.S. diet, and their degree of variation.

Three studies on diet and cancer are currently being developed. One is a large case-control study of breast cancer among women of Japanese descent now living in Hawaii and the West Coast. When Japanese women migrate to Hawaii and California, their low rates of breast cancer rise over a period of several generations. During this period of acculturation, there should be sufficient variation in diet among these women to separate and assess possible dietary risk factors for

breast cancer and to define the period of life during which diet is operative in promoting breast cancer risk. A second study, a prospective cohort study to be done in collaboration with the Kaiser Foundation Research Institute, will evaluate the relationship between low serum cholesterol and elevated risk of cancer. Within this reasonably representative and large American cohort of about 200,000 individuals, the possibility that preclinical cancer itself could be the cause of the low serum cholesterols, the spectrum of anatomic sites that are involved, and the relationship of serum cholesterol to both cancer incidence and prognosis will be assessed. Third, a case-control study of invasive cancer of the uterine cervix is being developed that will assess folate, vitamin A, carotene, and other micronutrient levels with both diet recall and biochemical techniques.

Publications:

Ziegler, R.G., Blot, W.J., Hoover, R., Blattner, W.A., and Fraumeni, J.F., Jr.: Nutritional factors and the low risk of colon cancer in Southern retirement areas: A study protocol. Cancer Res. (In press)

Pottern, L.M., Morris, L.E., Blot, W.J., Ziegler, R.G., Fraumeni, J.F., Jr.: Esophageal cancer among black men in Washington, D.C.: I. Alcohol, tobacco, and other risk factors. JNCI (In press)

Ziegler, R.G., Morris, L.E., Blot, W.J., Pottern, L.M., Hoover, R.N., and Fraumeni, J.F., Jr.: Esophageal cancer among black men in Washington, D.C. II. The role of nutrition. JNCI (In press)

Contracts in Support of this Project

Individual studies numbered 1, 2, 3, 4, 10, and 12 were supported under a contract with Westat, Inc. of Rockville, MD. and studies numbered 6, 7, and 8 were supported under a contract with ORI, Inc. of Silver Spring, MD. See research projects Nos. Z01-CP-04779-05-EEB and Z01-CP-04378-06-EEB for information concerning these support contracts.

ENVIRONMENTAL EPIDEMIOLOGY BRANCH

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Children's Hospital, Phila. N01-CP-91049	Late Effects of Treatment for Cancer in Childhood	1231
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CENTER FOR DISEASE CONTROL (Y01-CP-00500)

Title: Epidemiologic Studies of Cancer in Alaskan Natives

Contractor's Project Director: Anne P. Lanier, M.D.

Project Officer (NCI): William J. Blot, Ph.D.

Objectives: To study the incidence of cancer in Alaskan natives from 1974 onward and the mortality from cancer in Alaskan natives from 1960 to the present; to investigate several striking familial aggregations of cancer in Alaskan natives, particularly nasopharyngeal cancer and hepatocellular carcinoma; and to search for environmental determinants of nasopharyngeal cancer, with particular emphasis on the presence of nitrosamines in Alaskan preserved fish.

Methods Employed: Newly-diagnosed cancers and cancer deaths in Alaskan natives will be ascertained through the facilities of the Public Health Service Hospitals in which virtually all Alaskan natives receive their health care. The data will be collected in a format compatible with the SEER Registries to permit comparison with other U.S. populations. Cancer-prone families will be studied in collaboration with professionals from the Family Studies Unit of NCI's Environmental Epidemiology Branch, utilizing clinical and laboratory protocols tailored to the malignancies to be evaluated. This will include hepatitis B and Epstein-Barr virus assays, immunologic and genetic tests, and other serologic markers aimed at uncovering mechanisms of disease susceptibility. A case-control study of nasopharyngeal cancer is planned, which will focus on dietary exposures of possible etiologic interest. Samples of suspect native food items will be assayed for the presence of nitrosamines and mutagenic activity.

Major Findings: The updated cancer mortality and incidence statistics show that rates continue to be elevated among Alaskan natives for cancers of the nasopharynx, liver and gall bladder, kidney, and uterine cervix, and that the rates of lung, colorectal, and breast cancer are now approaching those of the U.S. white population. Laboratory analyses of selected food items, clinical investigations of high-risk families and planning for the case-control study all have begun.

Significance to Biomedical Research and the Program of the Institute: The projects in this proposal are directed toward investigating the unusual patterns of cancer in the culturally and geographically unique population that Alaskan natives represent and identifying risk factors for these cancers.

Proposed Course: Analyses of time trends in cancer incidence and mortality will continue. Procedures for biologic specimen collection from cancer-prone families will be implemented. A selected number of suspect food items will be submitted for nitrosamine analysis, and the case-control study will be initiated.

Date Contract Initiated: December 17, 1979

Current Annual Level: $145,523

CHAIM-SHEBA MEDICAL CENTER. ISRAEL (NO1-CP-01042)

Title: Radiation Risk Estimation in Israeli Children Irradiated for Tinea Capitis.

Contractor's Project Director: Baruch Modan, M.D.

Project Officer (NCI): John D. Boice, Jr., Sc.D.

Objectives: To determinine the incidence of cancer in 10,000 Israeli children irradiated for ringworm of the scalp, in 10,000 nonexposed persons selected from the general population and in 5,000 nonexposed siblings.

Methods Employed: The study cohorts were previously identified from immigration records (1949-60) and the risk of thyroid cancer evaluated. Medical records in all Israeli hospitals and records available in the Central Tumor Registry are being searched to determine malignant and benign tumors that have occurred in the exposed and comparison cohorts. Detailed dosimetry data are being abstracted. Death certificates will be obtained for those who have died and vital status as of 1981 will be determined for all enrolled persons. Sites of particular interest include: thyroid, brain, parotid gland, breast, bone, lung, esophagus, larynx, skin, leukemia, and lymphoma.

Major Findings: Thus far, 177,799 pathology records were reviewed, of which 7,953 (4.5%) were selected for further work-up. Listings of potential study subjects, to be matched with existing study rosters, have been prepared including patient names, father's name, year of birth, date of biopsy, department, hospital admission number, file number, and diagnosis site.

Significance to Biomedical Research and the Program of the Institute: The study of patients irradiated for benign diseases has been an important area for evaluating biological mechanisms for carcinogenesis in man. The minimal confounding effects of other carcinogenic influences such as smoking or occupation and the possible greater susceptibility of young people to environmental carcinogens enhance the chances of detecting radiogenic effects and provide an opportunity for life-time studies of cancer incidence. This particular study has already reported excess thyroid nodular disease following low-level exposure (9 rad) and further follow-up will be informative for clarification of this finding. This project has direct relevance to the Institute and Federal commitment to evaluate the adverse health effects of ionizing radiation.

Proposed Course: Date collection will proceed for approximately three years followed by analysis and publication of study findings.

Date Contract Initiated: September 25, 1980.

Current Annual Level: $130,000.

CHILDREN'S HOSPITAL, PHILADELPHIA (NO1-CP-91049)

Title: Late Effects of Treatment for Cancer in Childhood.

Contractor's Project Director: Anna T. Meadows, M.D.

Project Officer (NCI): John D. Boice, Jr., Sc.D.

Objectives: The primary objective is to evaluate the potential carcinogenic effects of various modalities (radiation and chemotherapy) used in the treatment of childhood cancers. Other objectives are to evaluate the interaction of therapy and genetic predispositions to cancer, determine incidence and histopathology of second cancers, and evaluate survival.

Methods Employed: Using the resources of the Late Effects Study Group, an international collaboration involving 13 major centers for childhood cancer therapy, a case-control study of approximately 200 individuals with second primary cancer is underway. Rosters of long-term survivors of childhood cancer have been prepared, controls have been randomly selected from this roster, therapy information is being abstracted from records, and detailed radiation dosimetry performed. The distribution of chemotherapeutic agents and radiation treatments between cases and controls will be compared.

Major Findings: Data collection is ongoing for the case-control study and is insufficient for analysis, therefore there are no major findings to report. Current progress has continued to be behind schedule. However, the influence of radiation and genetic predisposition on the risk of bone sarcoma has been evaluated, acute and long-term cytogenetic effects of treatment in childhood cancer reported, and the occurrence of "trilateral" retinoblastoma described.

Significance to Biomedical Research and the Program of the Institute: The study of the carcinogenesis effects of anti-tumor agents and radiation therapy should provide insights into the biological mechanisms of cancer etiology. The minimal confounding effects of other carcinogenic influences, such as smoking or occupation, and the possible greater susceptibility of young people to environmental carcinogens, enhance the chances of detecting increased risks due to therapy. Assessment of the carcinogenic potential of anti-tumor agents is part of the Institute's overall investigation of possible carcinogenic actions of therapeutic drugs.

Proposed Course: The above procedures will be continued. The medical records will be abstracted, radiation dose measurements performed, and the case-control study analysed and reported.

Date Contract Initiated: September 30, 1979.

Current Annual Level: $201,629.

CONTRACTOR TO BE SELECTED (P00022)

Title: Mesothelioma and Employment: A. A Case-control Study Utilizing Population-based Tumor Registries.

Contractor's Project Director: Not available.

Project Officer: Roger Connelly.

Objectives: To collect information to evaluate the role of occupational exposure in the origin of mesothelioma. Asbestos miners and insulators and shipyard workers are known to be at higher risk for mesothelioma, but information on other industries where exposures to asbestos and other fibrous products are lower is incomplete. This study is designed to fill this gap.

Methods Employed: The project has a case-control design. Cases of mesothelioma from tumor registries and their matched controls will be interviewed (next-of-kin will be interviewed if the cases are deceased) to obtain work histories and other information pertinent to the origin of this tumor.

Major Findings: Not applicable.

Significance to Biomedical Research and Program of the Institute: This contract will provide data needed to estimate the risk of low-level asbestos exposures in a variety of industries and occupations. The rising incidence of this tumor and the widespread exposure to asbestos underscores the need to identify new exposure groups so that preventive action may be taken.

Proposed Course: Award contract in August, 1981.

Date Contract Initiated: Not available.

Current Annual Level: Not available.

E. I. DUPONT DE NUMOURS & CO., INC. (N01-CP-01038)

Title: Study of the Dupont Chambers Works Bladder Cancer Screening Program

Contractor's Principal Investigators: Bruce W. Karrh, M.D.

Project Officer (NCI): Thomas J. Mason, Ph.D. and Philip C. Prorok, Ph.D.

Objectives: The primary objective of the study is to evaluate the effectiveness of urinary cytology as it is currently utilized in detecting early bladder cancer among employees at DuPont's Chambers Work facility.

Methods Employed: Certain data are abstracted from DuPont employee medical and employment records, including complete work histories, every urine cytology reading, the results of every urine blood test, information from physical exams, and information concerning the clinical course of bladder cancer among this work force.

Major Findings: This contract is in its data collection phase, and no analysis of data has as yet been undertaken.

Significance to Biomedical Research and the Program of the Institute: This study provides an extensive re-examination of the DuPont bladder cancer screening program as recommended by the State-of-the-Arts-Conference on Bladder Cancer Screening in December, 1977. Thus, it will aid NCI in formulating policy and reseach priorities with respect to bladder cancer screening and populations at high risk as a consequence of occupational exposure.

Proposed Course: It is anticipated that preliminary analysis will commence in September, 1981.

Date Contract Initiated: September 5, 1980

Current Annual Level: $25,000

EMORY UNIVERSITY (NO1-CP-81014)

Title: Collection of Interview Data to Assess Bladder Cancer Risk and Exposure Related to Textile Production

Contractor's Project Director: Margaret A. Child, M.D.

Project Officer (NCI): Robert N. Hoover, M.D.

Objectives: To investigate the role of exposure related to textile production in the etiology of human bladder cancer.

Methods Employed: All cases of bladder cancer diagnosed from January 1-December 31, 1979 in greater Atlanta were identified and controls were selected from the general population. The questionnaire elicited information about smoking, occupation, residence, drinking water, hair dyes, coffee, artificial sweeteners, drugs and medical history.

Major Findings: Data have been collected and edited. Analyses will be conducted in the Branch in the coming year.

Significance to Biomedical Research and the Program of the Insitute: This study addresses the possible role of exposures related to occupations in the textile industry in human bladder cancer. Although it has been suggested that people who have worked in the textile trades are at increased risk of bladder cancer, the existence and nature of such an association has not been fully explored. The investigation of possible occupational carcinogens is one of the Intitute's and the Branch's major concerns.

Date Contract Initiated: May 1, 1979

Current Annual Level: 0 (No additional funds)

GEORGE WASHINGTON UNIVERSITY (NO1-CP-81051)

Title: Study of Ovarian Cancer in Greater Washington, D. C.

Contractor's Project Director: Larry McGowan, M.D.

Project Officer (NCI): Robert N. Hoover, M.D.

Objectives: To investigate the role of exogenous hormones and other factors in the etiology of ovarian cancer.

Methods Employed: All cases of ovarian cancer being diagnosed in 25 hospitals in the greater Washington (approximately 13 per month) are being identified as they occur during the 3-year period August 1, 1978-July 31, 1981. For each case, a control is drawn of the same age and race, and discharge from the same hospital at approximately the same date. After the subject and her physician have given permission, the subject is interviewed in her home. The interviewer uses a questionnaire that elicits information about the few factors known to relate to risk of ovarian cancer (marital status, gravidity, age at first pregnancy, parity, infertility) and many factors under investigation (hysterectomy; use of menopausal estogens, oral contraceptives and other exogenous hormones; occupational exposures; family history of diseases; childhood illnesses.)

In addition to the personal interview, questionnaires mailed to subjects' physicians and independent review of the tumor tissue provide information for analysis. The mailed questionnaires permit us to verify exposures to exogenous estrogens. The independent slide reviewer is valuable because of the variety of ovarian cancer types, types that differ in cellular origin, histologic appearance, survival patterns, and risk factors.

Major Findings: The case and control ascertainment and interviewing portion of the survey are still ongoing and will be analyzed later.

Significance to Biomedical Research and the Program of the Insitute: The study directly addresses the possible role of exogenous hormones in ovarian cancer. These agents are widely used and have been linked to malignancies of reproductive organs, so it is imperative to examine their long-range effects on risk of cancer of other reproductive organs. In addition, any elucidation of the etiology of ovarian cancer would be welcome. Although this form of malignancy is relatively common (17,000 cases per year in the U.S.) and often fatal (10,000), very little is known of its causes.

Proposed Course: Data will be collected through 1981. The analyses will be performed in the Environmental Epidemiology Branch.

Date Contract Initiated: October 1, 1978.

Current Annual Level: $93,573.

HARVARD UNIVERSITY, CAMBRIDGE, MASSACHUSETTS (NO1-CP-81058)

Title: Follow-up of Fluoroscopically Examined Tuberculosis Patients in Relation to Incidence of Cancer.

Contractor's Project Director: Richard R. Monson, M.D.

Project Officer (NCI): John D. Boice, Jr.

Objectives: Approximately 10,000 former tuberculosis patients, treated between 1930-52, are being evaluated. Many have received pneumothorax and associated multiple chest fluoroscopies. The objectives are (1) to evaluate the risk of radiation-induced breast cancer in older women, (2) to evaluate the risk of radiogenic lung cancer and leukemia in men and women, (3) to evaluate the association between Isoniazid and liver disease, and (4) to evaluate the interaction between radiation and biological modifiers of risk.

Methods Employed: Records of former tuberculosis patients are available in Massachusetts and are being reviewed. Fluoroscopic exposures, medical treatments and demographic information have been abstracted. Follow-up activities include the use of HSPH Town Lists, Motor Vehicle Records, Department of Vital Statistics Records, outpatient records, and other sources. Questionnaires are being sent to persons known to be alive to ascertain current medical status. Death certificates are being obtained for all who have died. Analysis will be done using a general computer program. Permission and approval was obtained from the Massachusetts Commissioner of Public Health, Middlesex County Hospital, Essex Sanatorium, North Reading State Sanatorium, Norfolk County Hospital, Plymouth County Hospital and the State Archives (repository for early Rutland Sanatorium records). A multipurpose abstract form has been used to obtain fluoroscopic data, medical information, location information, and tracing information.

Major Findings: The abstraction of data is continuing. Death certificates have been obtained for persons who died in Massachusetts. The questionnaire has been sent to those patients who are living. The desired study size has been obtained, but no results are yet available.

Significance to Biomedical Research and the Program of the Institute: One of the National Cancer Institute's most controversial programs has been the Breast Cancer Detection and Demonstration Project (BCDDP) which has enrolled 280,000 women for annual mammographic x-ray examinations to detect breast cancer early in asymptomatic women. Guidelines for cancer screening and detection by mammography have been undergoing major changes, and it is urgently important that the guidelines be validated. Specifically, it is important to determine (1) whether women 35 to 49 years of age at screening are likely to develop radiation-induced breast cancer from repeated low-level exposures, and (2) whether women at high natural-risk of developing breast cancer because of underlying host conditions (such as benign breast disease or family history of cancer) are at especially high-risk of radiogenic breast cancer.

In addition, it is unknown whether repeated low-dose radiation exposures have the same effect as a single large exposure in inducing leukemia and lung cancer. Because population exposures are in large part to low doses, cumulated over many years, it is extremely important to estimate risks from irradiated populations receiving similar, though much larger, radiation exposures.

Proposed Course: Specific plans for the last year of this contract include (1) final mailings of questionnaires and computerization of results, (2) dosimetry evaluation, (3) analysis, and (4) final report.

Date Contract Initiated: September 1978.

Current Funding Level: $275,760.

HAWAII UNIVERSITY OF (NO1-CP-71006)

Title: Occupational Cancer Risk in Hawaii

Contractor's Principal Investigator: Ming Pi Mi, Ph.D.

Project Officer (NCI): Thomas J. Mason, Ph.D.

Objectives: This project is designed to provide data which will permit the linkage of official records: Civil Defense file, birth records, and death certificates for the population of Hawaii from 1942-78, with specific emphasis on occupation and/or place of employment.

Methods Employed: From a Civil Defense file with detailed information on the Hawaiian population of 1942-43, occupational data have been abstracted for the Oahu cohort, as well as on individuals residing in the remaining counties (islands of Kauai, Maui, Molokai, Lanai and Hawaii) during the war years. These data are important because of the number of individuals who resided and worked on sugar, pineapple, and rice plantations, and might have been exposed to carcinogens. Occupational data will be abstracted from all death certificates for the period in question.

Major Findings: The abstracting of occupation has been completed for all persons registered in 1942-43. Vital records files have been expanded to include all deaths through 1978. Linkage programs have been written, and the population of 1942-43 has been followed through 1978. We are currently pursuing both proportionate mortality and proportionate cancer mortality analyses for each of the major races with emphasis on occupation.

Significance to Biomedical Research and the Program of the Institute: The collection of occupational data fits in well with the specific interest in the geographic distribution of malignancy in the United States that the Environmental Epidemiology Branch has developed over the past several years. The ability to link records over this time span will greatly facilitate the investigation of occupational factors related to malignancy.

Proposed Course: The resultant record-linked data bases will permit the selection of a final occupational group of interest from death certificate data and the linkage of official records on these individuals through time, using birth records as interim checks for changes and Civil Defense records for an initial occupation.

Date Contract Initiated: April 11, 1977

Current Annual Level: $132,670

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (NO1-CP-11017)

Title: International Radiation Study to Evaluate the Risk of Radiation Exposure in Cervical Cancer--European Segment.

Contractor's Project Director: Allen Linsell, M.D.

Project Officer (NCI): John D. Boice, Jr., Sc.D.

Objectives: To quantify the risk of low-level radiation doses (under 100 rad) and to evaluate the influence of host factor (such as age) on subsequent radiogenic risk.

Methods Employed: More than 22,000 patients treated for cervical cancer in 22 European clinics will be evlauated for the occurrence of second cancers subsequent to radiotherapy. Organ doses to sites outside the pelvis will receive low-level doses (under 100 rad) and will be accurately characterized. Detailed dosimetry information will be abstracted from hospital records. Morbidity and mortality will be determined through active follow-up. Fifteen cancer registries have been conducting cohort analyses on the risk of second cancers among 200,000 former cervical cancer patients; case-control studies in these registry areas will be conducted to evaluate the influence of cancer risk factors, such as smoking, on subsequent risk, and to provide detailed dosimetry information for risk assessment.

Major Findings: The cancer registry cohort analyses are almost complete and will be published as a monograph near the end of this year. Preliminary findings suggest excess risks, related to radiation, of bladder cancer, rectal cancer, kidney cancer, ovarian cancer, corpus uteri cancer, and other cancers and lymphomas. A deficit of breast cancer possibly related to ovarian ablation was also observed.

Significance to Biomedical Research and the Program of the Institute: The project will estimate the risk of radiogenic cancers following low-dose irradiation. The project has the potential to elucidate mechanisms of carcinogenesis in general and is directly relevant to the Institute and Federal commitment to evlauate the possible adverse health effects of low-level ionizing radiation. Thus this project will provide information useful in formulating preventive measures and in setting radiation protection guidelines for occupational, medical, and public exposure to radiation.

Proposed Course: Data collection will proceed for approximately three years followed by analysis and publication of study findings. A monograph of the cancer registry cohort analyses should be published within the year.

Date Contract Initiated: April 11, 1977.

Current Annual Level: $109,610.

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (NO1-CP-71015)

Title: Feasibility Studies for the International Radiation Study of Patients Irradiated for Cervical Cancer--European Segment.

Contractor's Project Director: Jacques Esteve, Ph.D.

Project Officer (NCI): John D. Boice, Jr., Sc.D.

Objectives: To determine whether it is feasible to reactivate the European segment of an international study of cancer following radiotherapy, whether patients can be traced, and whether cancer registries in Europe can be included in the overall study. The goal is to quantify the risk of radiogenic cancers in those organs outside the pelvis that received low-level doses (under 100 rad).

Methods Employed: Feasibility evaluations have been made in 22 European clinics that participated in the former radiation study to determine the extent of radiotherapy details available in hospital records, the ease of patient follow-up, and the extent to which information on risk factors (smoking, etc.) is available in hospital records. The 22 clinics are in Austria, Czechoslovakia, Denmark, France, Federal Republic of Germany, Greece, France and the United Kingdom. European cancer registries have been contacted and preliminary cohort analysis conducted relating the risk of second cancers to radiation therapy received during previous treatment for cervical cancer.

Major Findings: Nineteen of 22 clinics are eligible for study and have agreed to collaborate, as have cancer registries in Denmark, Finland, Sweden, Norway, Slovenia, Yugoslavia, Canada, and England (5 registries). The expansion and extension of the international study appears feasible. Three Working Group meetings have been held and the proposed expansion delineated. Preliminary cohort analyses of cancer registry data suggest excess risk of solid cancers.

Significance to Biomedical Research and the Program of the Institute: The project will attempt to quantify the risk of radiogenic cancers following low-dose irradiation. The project has the potential to elucidate mechanisms of carcinogenesis in general and is directly relevant to the Institute and Federal commitment to evaluate the possible adverse health effects of low-level ionizing radiation. Thus it is relevant to prevention programs and the setting of radiation protection guidelines.

Proposed Course: The cancer registry cohort analyses will be reported and published in a monograph. The expanded study will be supported under contract NO1-CP-11017.

Date Contract Initiated: April 1980 (Modification to ongoing contract)

Current Annual Level: $35,000

IOWA, UNIVERSITY OF (NO1-CP-11020)

Title: A Study of Environmental Factors in the Origin of Leukemia and Non-Hodgkin's Lymphoma among Adult White Males from Rural Areas.

Contractor's Project Director: Peter Isacson, M.D.

Project Officer (NCI): Aaron Blair, Ph.D.

Objectives: To collect information to evaluate the role of environmental determinants (particularly agriculturally related) in the origin of leukemia and non-Hodgkin's lymphoma. This contract will supplement NO-CP-01022 by providing additional study subjects needed to obtain sufficient statistical power for this project.

Methods Employed: The project is a case-control design. Three hundred histologically confirmed cases for each tumor and 600 matched controls among adult white males will be selected and interviewed to determine personal habits and occupational exposures. Controls (meeting age, race, and sex requirements) will be randomly selected from the general population.

Major Findings: Project recently initiated; no reports have been received as yet.

Significance to Biomedical Research and the Program of the Institute: This contract will provide needed data on the origin of leukemia and non-Hodgkin's lymphoma. Geographic studies of these cancers by the Branch have suggested new leads, particularly in the area of farm-related exposures, that urgently need to be evaluated. The increase in non-Hodgkin's lymphoma in recent years and the limited effort devoted to the epidemiology of this cancer further underscores the need for this project.

Proposed Course: Continue with methodology as described above.

Date Contract Initiated: June 1, 1981.

Current Annual Level: $246,839

KAISER FOUNDATION RESEARCH INSTITUTE, OAKLAND, CALIFORNIA (NO1-CP-81047)

Title: Studies on Environmental Cancer Utilizing a Pre-Paid Health Plan.

Contractor's Project Director: Gary Friedman, M.D.

Project Officer (NCI): Robert N. Hoover, M.D.

Objectives: (a) To evaluate hypotheses concerning environmental causes of cancer by analysis of information in a Pre-Paid Health Plan which has been recorded over many years on large groups of patients having particular cancers, and to compare the data to those on individuals without the disease. (b) To follow up this analysis by extensive studies on those individuals who have had known exposures to the particular environmental factors which are suspect in the etiology of the cancers concerned. This is one of two Kaiser Foundation Research Institute collaborating contracts (see N01-CP-81046).

Methods and Major Findings: In Northern California the data collection for a case-control study of ovarian cancers similar to that in Portland, was completed. The protocol for a case-control study of patients who developed breast cancer after having had a bilateral oophorectomy was developed and data collection is nearing completion. The major exposure of interest being assessed in this study, in addition to the usual breast cancer indicators, is the use of menopausal estrogen replacement therapy. Both the Northern California and the Portland Plans have submitted information on malignancies occurring in the Health plans and various other demographic information concerning the potential hypotheses to be explored in these health plans. Extensive evaluation of cancer incidence among various industrial groups covered by the pre-paid plan has been done, and a number of analytic studies based on these evaluations are currently being designed. Two new evaluations were begun in this year. A study of the relationship of serum cholesterol levels among men attending a multiphasic screening exam and their subsequent risk of malignancy is underway. In addition, a case-control study of patients with leukemia and lymphoma was started which will evaluate the risk associated with diagnostic irradiation.

Significance to Biomedical Research and the Program of the Institute: The objective of the Environmental Epidemiology Branch is to generate and test ideas concerning the environmental and host determinants of cancer by a broad range of epidemiologic studies based on knowledge and application of clinical medicine and oncology, statistical methodology, new developments in carcinogenesis, and resources available at various levels. This project is immediately relevant to this broad objective because it attempts to focus directly on environmental factors which occur during an individual's life and to which he may be exposed at various time periods.

Proposed Course: This contract will terminate in FY81. A new project of studies of environmental cancer in pre-paid health plans will be initiated and this institution is expected to respond to the RFP for that project. Evaluations to be conducted in the first year of the new project include evaluation of the interrelationships between oral contraceptive use and benign and malignant breast disease, drug use and reproductive risk factors for endometrial cancer, and follow-up studies based on findings from the cholesterol, occupational and radiation studies currently being conducted or analyzed.

Date Contract Initiated: August 1, 1978.

Current Annual Level: $240,232.

KAISER FOUNDATION RESEARCH INSTITUTE, PORTLAND OREGON (NO1-CP-81046)

Title: Studies on Environmental Cancer Utilizing a Pre-Paid Health Plan

Contractor's Project Director: Andrew Glass, M.D.

Project Officer (NCI): Robert Hoover, M.D.

Objectives: (a) To evaluate hypotheses concerning environmental causes of cancer by analysis of information in a Pre-Paid Health Plan which has been recorded over many years on large groups of patients having particular cancers, and to compare the data to those on individuals without the disease. (b) To follow up this analysis by extensive studies on those individuals who have had known exposures to the particular environmental factors which are suspect in the etiology of the cancers concerned. This is one of two Kaiser Foundation Research Institute collaborating contracts (see NO1-CP-81047).

Major Findings: Computerization of the tumor registry from 1969 forward has been completed and time-trend analyses are underway. The data collection phase of a case-control study of ovarian cancer has been completed. For the ovarian cancer study a common protocol has been worked out between the Portland region and Northern California region under the direction of the project officer at NCI. The focus of this study is on therapeutic drugs and medical conditions which alter the pituitary-ovarian hormonal axis. Data on stage of disease and survival information have been abstracted and computed for the series of breast cancer patients included in a prior study done in this plan. This information has been forwarded to NCI. Case-control studies of cholesterol level and risks of colon and lung cancer in men, and diagnostic irradiation and the risk of leukemia and lymphoma, have been initiated and are in the data-collection phase.

Significance to Biomedical Research and the Program of the Institute: The objective of the Environmental Epidemiology Branch is to generate and test ideas concerning the environmental and host determinants of cancer by a broad range of epidemiologic studies based on knowledge and application of clinical medicine and oncology, statistical methodology, new developments in carcinogenesis, and resources available at various levels. This project is immediately relevant to this broad objective because it attempts to focus directly on environmental factors which occur during an individual's life and to which he may be exposed at various time periods.

Proposed Course: This contract will terminate in FY 81. A new project of studies of environmental cancer in pre-paid health plans will be initiated, and this institution is expected to respond to the RFP for that project. Evaluations to be conducted in the first year of the new project include evaluations of the interrelationships between oral contraceptive use and benign and malignant breast disease, drug use and reproductive risk factors for endometrial cancer and follow-up studies based on findings from the cholesterol, occupational and radiation studies currently being conducted or analyzed.

Date Contract Initiated: August 1, 1978.

Contract Annual Level: $293,389.

KAISER FOUNDATION RESEARCH INSTITUTE, OAKLAND, CALIF. (NO1-CP-91028)

Title: Relationship Between Menopausal Estrogens and the Risk of Breast Cancer Among Oophorectomized Women.

Contractor's Project Director: Harry K. Ziel, M.D.

Project Officer (NCI): Robert N. Hoover, M.D.

Objectives: To investigate the relationship of menopausal estrogens to risk of breast cancer among women who had an oophorectomy.

Methods Employed: The surgical record books from the Southern California Permanente Medical Group from 1952 through 1970 were reviewed manually to identify a group of health plan members who had oophorectomy during this time period. These lists were compared with a list of breast cancer patients identified by the health plan's tumor registry during the years 1972-1977 in order to identify patients with a history of oophorectomy who subsequently developed breast cancer. From the same surgical record books and the files of health plan members, four controls for each case were drawn. These controls are women having undergone an oophorectomy in the same year as the case, matched on age at which this operation occurred, and duration of health plan membership (to the date of diagnosis of the case). Thus far, 85 cases have been identified and their health plan records abstracted; 255 control women have also been identified and their records abstracted. An additional study of estrogens and other risk factors as they relate both to breast cancer and abnormal mammographic findings utilizing data from a large screening program in this plan is also being conducted. The potential teratogenicity of estrogens is also being evaluated via a case-control evaluation of drug exposures during pregnancy and subsequent development of limb-reduction and cardiac defects in the fetus.

Major Findings: Data collection is nearing completion therefore no formal findings are available.

Significance to Biomedical Research and the Program of the Institute: The study directly addresses the role of exogenous hormones in a group of women whose risk of breast cancer has been lowered by oophorectomy and whose likelihood of using menopausal estrogens has been raised. These factors will enhance the chances of detecting and increase in risk. Assessment of the carcinogenic potential of exogenous estrogens is part of the Institute's overall investigation of possible carcinogenic actions of therapeutic drugs.

Proposed Course: The clinical records of all the cases and matched controls will continue to be reviewed and information concerning exposure to estrogens and other pertinent risk factors prior to the date of diagnosis of the case will be abstracted and computerized. The investigators at Southern California will analyze their own data and in addition will send a copy of the material to NCI so that the data can be merged with similar information which will have been obtained from two other Kaiser Permanente Health Plans. This contract will terminate in FY-81. A new project of studies of cancer in pre-paid health plans will be initiated will respond to that RFP.

Date Contract Initiated: July 1, 1979.

Current Annual Level: $47,309.

LOUISIANA STATE UNIVERSITY (NO1-CP-91023)

Title: Cancer in Southern Louisiana: A Case-Control Study of Lung, Pancreas, and Stomach Cancers

Contractor's Project Director: Pelayo Correa, M.D.

Project Officer (NCI): Linda W. Pickle, Ph.D.

Objectives: To identify risk factors responsible for the high rates for lung, pancreas, and stomach cancers in southern Louisiana.

Methods Employed: A case-control interview study among residents of southern Louisiana parishes will provide information on lifetime histories of residence, occupation, tobacco and alcohol consumption, diet, and ethnic and social factors for approximately 3,000 cancer cases and controls.

Major Findings: Sample identification and interviewing of cases and controls is under way.

Significance to Biomedical Research and the Program of the Institute: This investigation generates data to uncover reasons for the exceptional occurrence of cancer in Louisiana and thus provide information useful in formulating measures aimed at prevention.

Proposed Course: It is anticipated that interviewing of lung cancer cases and controls will be completed by July 1, 1981, while interviewing of the stomach and pancreas cases and controls will continue until 1982. Analysis will be completed by May 1, 1982.

Date Contract Initiated: March 13, 1979

Current Annual Level: $252,520

MAYO FOUNDATION (NO1-CP-01057)

Title: Leukemia Following Chemotherapy for Ovarian Cancer

Contractor's Project Director: George D. Malkasian, M.D.

Project Officer (NCI): Mark H. Greene, M.D.

Objectives: Identify, abstract, and follow up approximately 1,500 one-year survivors of ovarian cancer to document the occurrence of second tumors, particularly leukemia. Quantify the risk of subsequent malignancy in relation to the antineoplastic treatment employed for the ovarian cancer.

Methods Employed: Suitable cases will be culled from 6,000 women with ovarian cancer treated at the Mayo Clinic between 1950 and 1979. Data will be abstracted from hospital records using an instrument developed by NCI/EEB. Data collection will focus on a detailed summary of ovarian cancer treatment. All patients will be actively followed to identify those who develop second cancers. All leukemia cases will be reviewed by an independent pathology panel. Death certificates will be sought on all deceased patients. The data will be pooled with identical data being collected from three additional sources (the M. D. Anderson Hospital, the Gynecologic Oncology Group, and the Princess Margaret Hospital) to evaluate in detail (a) the relation between ovarian cancer treatment and leukemia risk; (b) possible dose-response relationships; (c) possible chemotherapy-radiation therapy interactions in leukemia risk; (d) possible differences in leukemia risk of different chemotherapeutic agents; and (e) whether a leukemia-prone subset of ovarian cancer patients can be identified.

Major Findings: This contract is only six months old. There are no data yet available. The data collection is proceeding smoothly.

Significance to Biomedical Research and the Program of the Institute: This project is the linchpin of the NCI/EEB Late Effects of Cancer Therapy Program, which is designed to evaluate the potential carcinogenic effects of various modalities used in cancer treatment. One of the main goals of this project is to identify specific agents which are particularly safe or hazardous. Considerable data suggest that melphalan, a first line agent for ovarian cancer, is leukemogenic in man. Cyclophosphamide is equally effective clinically, but has not been systematically studied. The Mayo Clinic is the only institution known to have used single agent cyclophosphamide extensively in the treatment of ovarian cancer. This will permit a comparison of this agent's late effects with those of melphalan. These studies will also elucidate mechanisms of carcinogenesis in general.

Proposed Course: The data will be collected by the contractor over the course of one year. The data processing and analysis will be done by NCI/EEB staff under the supervision of the Project Officer.

Date Contract Initiated: September 30, 1980

Current Annual Level: $97,000

MEMORIAL SLOAN-KETTERING CANCER CENTER (NO1-CP-01050)

Title: Cell Proliferation and Susceptibility to Cancer of the Large Intestine

Contractor's Project Director: Martin Lipkin, M.D.

Project Officer (NCI): William A. Blattner, M.D.

Objectives: This project was established to explore the possible utility of an *in vitro* cell proliferation assay involving colon biopsies in an epidemiologic context. Various high- and low-risk groups from specified age groups are to be studied.

Methods Employed: Colonic biopsies are to be collected by the contractor on various well-defined risk groups. These are to be placed in short-term tissue culture and studied by radioautography. Raw data are to be entered into a computer for statistical analysis.

Major Findings: This is the first year of a two-year study, so the majority of effort has focused on specimen collection and on developing more sophisticated statistical approaches. These approaches have been applied to groups of patients with positive family histories and an improved discriminant for distinguishing between normal and high-risk patients has been developed. Using this statistical approach, high-risk individuals have been evaluated and the distribution compared to that expected if risk is 50 percent for an individual high-risk patient. A manuscript describing these results is in preparation.

Significance to Biomedical Research and the Program of the Institute: The assay procedure being studied under this procurement may represent a final common pathway in the pathogenesis of the malignantly transformed colon cancer cell. The abnormalities detected by this procedure mirror those seen in experimental models where carcinogens induce similar changes in rat colons. Characterization of the factors that modulate this phenotype in humans may help validate this test as a tool for studying human colon cancer carcinogenesis.

Proposed Course: During the second year of this two-year procurement, additional subjects will be biopsied based on results of the analysis of high- and low-risk groups currently under way. A medical and dietary questionnaire will be coded and keyed to assess the effects of these factors on test results.

Date Contract Initiated: September 26, 1980

Current Annual Level: $98,000

MINNESOTA UNIVERSITY OF (N01-CP-43384)

Title: Immunodeficiency -- Cancer Registry

Contractor's Project Director: John H. Kersey, M.D.

Project Officer (NCI): Robert N. Hoover, M.D.

Objectives: To study the frequency and possible determinants of malignancy in immunodeficient patients.

Methods Employed: Through the cooperation and good will of immunologists in many of the specialized immunology centers throughout the world, clinical information and occasionally biological specimens from immunodeficient patients who have developed malignancy are sent to the registry for tabulation and analysis. In the past year, continued monitoring for new cases of malignancy in patients with genetically determined immunodeficiency syndromes has gone on. In addition, selected clinical and pathological

material, particularly data on the surface marker characteristics of malignant lymphoblast cells in these patients has been analyzed. The registry has participated in the development of a large pool of individuals with one immunodeficiency disease, Wiskott-Aldrich syndrome. The emphasis in this study is to identify families who carry the gene for this syndrome and to study in some detail the characteristics of risks of the individuals with this syndrome as well as the carriers of the gene. In addition, a detailed description of the malignancy experience of patients with ataxia-telangiectasis has been performed.

Major Findings: To date 349 cases have been reported to the registry. Lymphorecticular malignancies predominate in almost all of the Genetically Determined Immunodeficiency Disease categories. Other tumors that appear to be excessive include stomach cancer (in adults only), malignant melanoma, soft tissue sarcoma, and hepatobiliary carcinomas. A special study of Wiskott-Aldrich families indicated a similar overall attack rate among the carriers of the gene and non-carriers, but an earlier age-at-onset among the carriers (nine years). Computerization of the registry information has been completed.

Significance to Biomedical Research and the Program of the Institute. Patients with altered immunologic states have demonstrated excess risks of malignancy several hundred times that of the general population. Study of the determinants of these excesses has a high probability of shedding light on immunologic aspects of cancer etiology.

Proposed Course: Case findings will continue to assure a continuing increase in the amount of material available for analysis in the registry. The analysis of the study of the Wiskott-Aldrich families will be expanded and the data will be analyzed to estimate a number of relative risks according to the presence of certain clinical characteristics. A case-control study of Wiskott-Aldrich patients who have developed malignancy will be instituted in order to test the hypothesis that the risk of malignancy may be related to the amount of immunostimulation experienced by these children. Possibilities of converting this contract to a grant will be explored during the coming year.

Date Contract Initiated: July 1, 1974

Current Annual Level: $111,000

MINNESOTA UNIVERSITY OF (NO1-CP-91014)

Title: Long-Term Mortality Study of Minnesota Iron-Ore Miners

Contractor's Project Director: Leonard M. Schuman, M.D.

Project Officer (NCI): William J. Blot, Ph.D.

Objectives: To clarify the role of iron and its compounds in human cancer by studying the mortality experience of iron-ore miners in the Mesabi Range, Minnesota where competing exposures from other environmental agents are minimal or nonexistent.

Methods Employed: Employee records of iron-ore mining companies were reviewed to assemble a large cohort of hourly-wage employees involved in hematite mining prior to 1965. General employment data were abstracted. Vital status of each member of the cohort will be determined and a death certificate obtained, if deceased.

Major Findings: A cohort of 13,000 iron-ore miners has been assembled and relevant work histories abstracted. Follow-up for vital status is nearing completion. Preliminary analyses indicate no overall excess of lung cancer among the total cohort. Evaluations of risk among subgroups of workers are underway.

Significance to Biomedical Research and the Program of the Institute: Studies of hematite miners in Great Britain have shown them to be at increased risk of dying from respiratory cancer; however, it is not clear whether iron-containing materials are the causative agents since other potentially carcinogenic exposures were present. This project is intended to clarify the role of iron and its compounds.

Proposed Course: Following completion of the follow-up for mortality, analyses will be undertaken comparing age-cause-specific mortality rates to rates from Minnesota and U.S. populations and examining risk associated with exposure to hematite dust. Hypotheses for further case-control or other studies will be developed.

Date Contract Initiated: July 1, 1979

Current Annual Level: $62,789

MINNESOTA, UNIVERSITY OF (NO1-CP-01030)

Title: Risk of Cancer in X-Ray Technologists.

Contractor's Project Director: Leonard M. Schuman, M.D.

Project Officer (NCI): John D. Boice Jr., Sc.D.

Objective: To evaluate the long-term effects of chronic exposure to radiation experienced because of occupation in 170,000 registered American radiological technologists.

Methods Employed: Using the resources of the American Registry of Radiologic Technologists, inactive members (30,000) will be located to determine vital status and cause of death, and living members will be contacted by mail questionnaire to determine cancer incidences and to obtain information on the use of dosimeters and cancer risk factors such as smoking history.

Major Findings: A feasibility study was completed which determined that inactive members of the society could be located and that it was possible to characterize radiation exposure based on length of employment, film badge readings, and questionnaire responses.

Significance to Biomedical Research and the Program of the Institute: The study will evaluate the effects of low-dose fractionated exposures received over a period of many years in a large group of occupationally exposed women. The two most sensitive organ sites for radiation carcinogenesis in women, the breast and thyroid, will be the focus of this investigation. This project is an important part of the overall Federal response to establishing a broad-based program in radiation carcinogenesis and has direct relevance to cancer etiology and the setting of radiation protection guidelines. This study is unique in that the average doses will be relatively low (5-15 rads) and extrapolation from high-dose data to estimate low-dose risks will not be necessary.

Proposed Course: Data collection will proceed for approximately 2 1/2 years followed by analysis and publication of findings. Afterwards, selected technologists will be sent mail questionnaires at 5-year intervals, and the National Death Index will be used for mortality follow-up.

Date Contract Initiated: Approximately September 1, 1981.

Current Annual Level: Approximately $250,000

MINNESOTA, UNIVERSITY OF (NO1-CP-01033)

Title: A Study of Environmental Factors in the Origin of Leukemia and Non-Hodgkin's Lymphoma among Adult While Males from Rural Areas.

Contractor's Project Director: Leonard Schuman, M.D.

Project Officer (NCI): Aaron Blair, Ph.D.

Objectives: To collect information to evaluate the role of environmental determinants (particularly agriculturally related) in the origin of leukemia and non-Hodgkin's lymphoma.

Methods Employed: The project is a case-control design. Three hundred histologically confirmed cases for each tumor and 600 matched controls among adult white males will be selected and interviewed to determine personal habits and occupational exposures. Controls (meeting age, race, and sex requirements) will be randomly selected from the general population.

Major Findings: This contract was initiated September 30, 1980, and insufficient time has elapsed for major findings to become available. Since the award hospitals have been contacted and their cooperation obtained, data collection instruments have been developed and selection of cases and controls is underway.

Significance to Biomedical Research and the Program of the Institute: This contract will provide needed data on the origin of leukemia and non-Hodgkin's lymphoma. Geographic studies of these cancers by the Branch have suggested new leads, particularly in the area of farm-related exposures, that urgently need to be evaluated. The increase in non-Hodgkin's lymphoma in recent years and the limited effort devoted to the epidemiology of this cancer further underscores the need for this project.

Proposed Course: The expiration date is September 29, 1983 and should continue to that date. During FY82, approximately 500 cases and 500 controls will be identified and interviewed.

Date Contract Initiated: September 29, 1980.

Current Annual Level: $192,569.

NATIONAL ACADEMY OF SCIENCES - (NO1-CP-01012)

Title: Epidemiologic Studies of Cancer Among A-bomb Survivors.

Contractor's Project Director: Dr. Hiroo Kato

Project Officer (NCI): Charles E. Land, Ph.D.

Objectives: The objectives of this collaborative study are to identify and quantify the possible interactive roles of radiation and other environmental and host risk factors in the development of certain cancers, and to carry out other studies of cancer risk among members of the A-bomb survivor population.

Methods Employed: Investigations based on the Life Span Study sample of 82,000 A-bomb survivors and 26,000 non-exposed, and a clinical subsample of 12,000 survivors and controls, are carried out at the Radiation Effects Research Foundation (RERF) in Hiroshima and Nagasaki, Japan. All studies involving new or unpublished data are collaborative, and include investigators from NCI, RERF, and outside organizations as required; collaboration is facilitated by personnel exchanges between RERF and at NCI. Methods include cohort studies of cancer incidence as determined from death certificates, tumor and tissue registries, and searches of hospital and clinical records, and case-control studies in which epidemiological factors other than radiation, as determined from existing records or by interview, are investigated. Reviews of diagnostic material by panels of pathologists may be employed in connection with studies.

Major Findings: Difficulties in the wording of the subcontract between the National Academy of Sciences and RERF were resolved, and funding for expenses incurred by RERF was begun in December 1980. This allowed a major increase in the interviewing effort for the first collaborative study undertaken under the contract, a case-control study of possible interactive effects of radiation and other factors in the causation of female breast cancer. Except for new cases, and controls, to be included based on a new breast cancer incidence study covering the period 1950-1980, interviewing was essentially complete as of July 1, 1981, and data were being coded and keypunched for a preliminary analysis. In addition to interview information obtained from living cases and controls, information on many variables was obtained by a search of RERF records for deceased cases and their matched controls. This phase of the study is expected to be completed before interviewing of the additional living cases and controls has been finished.

Agreement was reached to conduct an updated breast cancer incidence study at RERF, to begin in July 1981. The case finding will be under the supervision of Dr. Masayoshi Tokunaga, a pathologist at Kagoshima University Medical School who will be on leave from his faculty position while conducting this research in Hiroshima and Nagasaki, and who is expected to spend several months at NCI during the analysis and report-preparation phases of the study. Dr. Tokunaga played a similar role in the previous breast cancer incidence survey, covering the period 1950-1974.

Concurrently with his work on the breast cancer incidence study, Dr. Tokunaga will do serial sections of breast tissue material from the RERF autopsy series. This study will investigate the hypothesis, raised at the meeting in Hiroshima in 1979 of an NCI-RERF breast cancer pathology review panel, that non-neoplastic changes in breast tissue may be associated with radiation dose.

Questionnaire design for a case-control study of lung cancer incidence in relation to radiation dose and other risk factors, including smoking, occupation, and diet was carried out in Hiroshima and Bethesda.

Significance to Biomedical Research and the Program of the Institute: The project should substantially contribute to our knowledge of whether and to what extent radiation may interact with other known risk factors in inducing certain cancers. Dose-response relationships, and the influence of such factors as age at exposure and at observation for risk on the response to radiation, should be clarified by more intensive investigation of certain established cancer effects. The existence of the collaborative agreement permits great flexibility in initiating investigations of theoretical and practical interest to the NCI program in the epidemiology of radiation carcinogenesis that probably would receive much lower priorities in the RERF research program.

Proposed Course: Analyses of epidemiological case-control data and incidence data from the two breast cancer studies should be completed within the next year. Data collection should begin for the lung cancer case-control study. Investigations into the feasibility of a new case-control study of thyroid cancer, including a pathology review of existing diagnostic material, will be made. A study involving hormonal assays of stored blood samples from members of the clinical subsample who later developed cancers of the breast, thyroid, or prostate will be discussed.

Date Contract Initiated: November 10, 1979.

Current Annual Level: $227,835.

NATIONAL ACADEMY OF SCIENCES (NO1-CP-53573)

Title: Epidemiologic Studies in Etiology of Cancer in Veterans.

Contractor's Project Director: Seymour Jablon, M.A.

Project Officer (NCI): John D. Boice, Jr., Sc.D.

Objectives: To develop and conduct a broad program of epidemiologic studies among veterans.

Methods Employed: The Epidemiology Branches of the National Cancer Institute and the Medical Follow-up Agency of the National Academy of Sciences have developed an epidemiology program designed to make efficient use of the military-veteran population, utilizing medical, demographic, and environmental observations of veterans ascertained through facilities of the Veterans Administration and supplemented by mortality data. During the past year efforts have focused on completion of follow-up studies of veterens who have certain conditions (e.g., epididymitis) that may influence the risk of cancer. Also completed is a study of occupational exposure to tetrachloroethane, a chlorinated hydrocarbon used for impregnating clothing against mustard gas during World War II. Evaluation of the large Dorn study on smoking regarding occupational risk was initiated, as was the study of adjuvant drug carcinogenesis in VA clinical trails.

Major Findings: A study of patients treated with low-dose adjuvant chemotherapy for colorectal cancer revealed no measurable carcinogenic effect following thio-TEPA or FUdr exposures, although an inverse relationship between the occurrence of second primary cancer and age at diagnosis, irrespective of therapy, was suggested (JNCI 64: 501-511, 1980). A survey of men serving in chemical processing companies during WWII revealed no excess mortality from cancer that could be attributed to tetrachlorethylene or dry cleaning solvents that have been carcinogenic in laboratory animals.

Significance to Biomedical Research and the Program of the Institute: The Environmental Epidemiology Branch, NCI, is concerned with studies to identify and clarify environmental and host factors in cancer. Evaluation of the risk of developing second cancers following low-dose adjuvant chemotheraputic exposures has important implications regarding the NCI cancer therapy program, and may also provide insights into the mechanism of carcinogenesis. The VA-Surgical Oncology Group is a unique resource in this regard. The evaluation of the large veteran population in the Dorm study will be beneficial to the occupational studies program due to the unique combination of detailed data on smoking, occupation, and industry. The objectives can be more readily achieved by coordinating efforts with the unique resources and competent staff or the Medical Follow-up Agency.

Proposed Course: In this contract year, a survey will be made of cancer mortality among 300,000 U.S. veterans by occupational and smoking habits, updating the follow-up of the cohort originally assembled by Dorm. A study of testicular cancer is continuing to evaluate various risk factors including mumps, orchitis, and genitourinary defects. The evaluation of the risk factors of developing second cancers following adjuvant chemotherapeutic drugs will continue in 9 selected clinical trials.

Date Contract Initiated: June 28, 1971.

Current Annual Level: $265,000.

NATIONAL CENTER FOR HEALTH STATISTICS (YO1-CP-10503)

Title: Follow-up of the Health and Nutrition Examination Survey Cohort (HANES)

Contractor's Project Director: Ms. Helen Barbano

Project Officer (NCI): Aaron Blair, Ph.D.

Objectives: To follow up and interview the national sample of approximately 14,000 examinees who were 25 years or older at the first survey, in order to assess their current health status. These data will be extremely valuable in evaluating cancer/diet hypotheses.

Methods Employed: The contractor will trace all members of the cohort to determine their vital status. If deceased, death certificates will be obtained. Interviews will be conducted of survivors and next-of-kin of decedents, probing for dietary habits, occupational history, medical history, and other pertinent factors. These data will be coded, edited, and computerized, and made available to NCI for analysis.

Major Findings: Not applicable at this time.

Significance to Biomedical Research and the Program of the Institute: NCI is vitally interested in the role of nutritional factors in the origin of cancer. This project provides a unique opportunity to evaluate the role of diet in cancer development in a population. The availability of dietary data on study subjects obtained before the clinical onset of cancer eliminates the problems of recall bias that may occur in case-control studies.

Proposed Course: Continue what is described above under Methods.

Date Contract Initiated: June 18,1981.

Current Annual Level: $100,000

NAVAL MEDICAL RESEARCH INSTITUTE (YO1-CP-00502)

Title: Immunologic and Immunogenetic Studies of High-Risk Cancer Families and Logistical Support Services

Contractor's Project Director: Douglas Michael Strong, Ph.D.

Project Officer (NCI): William A. Blattner, M.D.

Objectives: This project was established to promote studies of the role of immunologic and immunogenetic factors in the etiology of cancer. As part of this effort, support is provided for maintaining a large repository of sera, plasma, white blood cells, and other biological materials obtained on members of high-risk families since 1974.

Methods Employed: Biologic specimens on high-risk families are processed for cryopreservation. When materials on all members of a single family or group of families are collected, specific immunologic studies are performed according to written protocol. Methods include HLA and B-cell alloantigen typing, PLT and MLC typing, in vitro immune studies of proliferative response, assays of regulatory cell function, natural killer cell activity, and quantification of subpopulations of immunoregulatory populations utilizing a fluorescence activated cell sorter (FACS-II).

Major Findings: The interagency agreement established in July, 1980 has focused major attention on establishing the laboratory, purchasing equipment, and implementing and standardizing assay systems during the last year. Initial efforts were hampered because of hiring limitations imposed at critical times by the Department of Defense, but recently the full complement of staff has been hired. Despite these limitations, several major tasks have been accomplished: (1) Large quantities of human T-cells from homozygous human donors were grown up and sent to Dr. Jack Strominger, who is extracting the DNA from these cells for gene cloning experiments aimed at extracting major histocompatibility genes. As a by-product, cell surface membrane preparations are being returned to the laboratory for use in attempting the establishment of hybridoma antibodies against Dr antigens. (2) Cell strains infected and not infected with a new human T-cell lymphoma virus were typed for HLA and B-cell alloantigens. Those cells known to contain the virus are untypable for HLA, while cell strains from the same patient are reproducably typable. HLA-D typing is not affected by the presence of the virus, helping to confirm the genetic source of the various cell strains. The scrambling of HLA cell surface gene products by the virus, causing a nonsense antigen to be produced, may be related to the mechanism by which the virus-infected cells are successfully propagated in the host. These cells, bearing altered self-antigens, may accumulate in an abnormal way because the usual cell surface markers are unrecognized by the host. (3) A familial chronic lymphocytic leukemia cluster was studied using the FACS-II. Previously-unrecognized cell surface markers, shared in a number of cases, were identified; and a case previously known to have chronic lymphocytic leukemia (CLL) appears to have reverted to normal by cell surface analysis. One sibling successfully treated for lung cancer has an altered percentage of suppressor cells. In vitro assessment of this abnormality is planned.

Samples have been collected on a number of families, including one with hairy cell leukemia, one with CLL, multiple cases of unexplained lymphocytosis, and one with multiple cases of non-Hodgkin's lymphoma and unexplained immunoglobulin abnormalities in close relatives. Protocols for study of these families have been laid out and will be implemented over the next several months.

Significance to Biomedical Research and the Program of the Institute: This interagency agreement represents the only institute research project specifically targeted at evaluating immunogenetic factors in human neoplasia. In addition, the immunogenetic program provides support for a large segment of NIH research aimed at studying HLA and disease associations, especially in terms of newly-defined antigen systems such as MT and MB.

Proposed Course: During the next year, we anticipate that research performed under the current agreement will proceed at a much more rapid pace since personnel and equipment problems have been solved. Specimens are now available on a number of families that will allow rapid implementation of several currently developed protocols.

Date Contract Initiated: June 20, 1980

Current Annual Level: $505,000

NEW JERSEY, STATE OF, DEPARTMENT OF ENVIRONMENTAL PROTECTION (NO1-CP-91048)

Title: Environmental Health Data Base for New Jersey

Contractor's Project Director: Thomas A. Burke

Project Officer (NCI): Thomas J. Mason, Ph.D.

Objectives: To develop an environmental data base for New Jersey which will be utilized in their ongoing intramural research projects.

Methods Employed: The water supply questionnaire developed by NCI will define the basic water quality information to be obtained for each purveyor in New Jersey. Records of DEP will be examined to determine how much of the needed information is on record, and all relevant data will be abstracted. Information not available from DEP records will be acquired through personal interviews with representatives of water purveyors and through examination of purveyors' records. Additional information on toxic substances and carcinogens in raw and finished water will be provided by the Program on Environmental Carcinogens and Toxic Substances. Completed questionnaires will be forwarded to NCI for coding and keypunching. Additional information not covered by the questionnaire will be coded and keypunched by Rutgers University and forwarded to NCI on computer tapes.

Major Findings: A total of 307 NCI Water Supply Data Abstracting Forms were completed, covering all water purveyors serving over 1,000 people. This amounts to coverage of over 95% of the New Jersey population. Information collected on these forms supplied the foundation for the drinking water section of the data base project. This information includes: (a) definition of service areas, population served, and raw water sources for each purveyor; (b) determination of historical and current chlorination and treatment practices; and (c) identification of potential raw water pollution sources, including all upstream dischargers.

Altogether 304 of the purveyors were sampled for volatile organic compounds including the trihalomethanes and heavy metals. Metal analysis was performed by the EPA laboratory in Cincinnati, Ohio. Analysis for organic compounds was performed in Cambridge, Massachusetts. This sampling

has provided NCI and New Jersey DEP with the following benefits: (a) quantitative water quality information on the drinking water of over 95% of the New Jersey population; (b) a better understanding of the relationship between chlorination practices and the amount of trihalomethane in finished drinking water; and (c) a better understanding of the relationship between water source pollution and finished water quality.

A twelve month subcontract was initiated with Rutgers University Department of Geography to assist in the compilation of the drinking water information. Rutgers was responsible for conducting all sampling and collecting current and historic water quality information which could not be obtained from the records of DEP.

All sampling and questionnaire information was submitted to the National Cancer Institute for computerization. A computer tape containing all the information will be returned to New Jersey for inclusion in the data base.

An investigation of seasonal variation of trihalomethanes in 15 selected drinking water supplies was conducted. The raw, treated, and delivered water of each supply was sampled at three different times over a six month period to measure variation. To ensure laboratory accuracy, samples were split and analyzed by the ERCO Labs and the DEP consultant laboratory at Rutgers. Results of this investigations will provide DEP and NCI with the following benefits: (a) a better understanding of the role of chlorination in the formation of trihalomethanes; (b) a better understanding of the relationship between raw water quality and delivered water; (c) an indication of the effectiveness of treatment in removing organic contaminants from drinking water; and (d) an indication of the tempered and seasonal variation in trihalomethanes in public drinking water supplies.

Significance to Biomedical Research and the Program of the Institute: This project will provide needed specificity concerning the exposure of persons in areas of the United States which have high rates of cancer mortality, and in which water is a suspect as a source of carcinogenic chemicals.

Proposed Course: The methodology described herein will continue. The data collected will be made available to the NCI.

Date Contract Initiated: September 30, 1979

Current Annual Level: $165,000

NEW JERSEY, STATE OF, DEPARTMENT OF HEALTH (NO1-CP-61031)

Title: Etiologic Studies of Cancer in New Jersey

Contractor's Project Director: Ronald Altman, M.D.

Project Officer (NCI): Thomas J. Mason, Ph.D.

Objectives: To examine the cancer mortality experience in the State of New Jersey with specific emphasis on quantifying risk factors for bladder cancer and lung cancer, as well as other anatomic sites with known or suspected occupational risk factors.

Methods Employed: (A) Descriptive studies - A system of computer programs has been developed which with modification can efficiently calculate sex and race-specific rates for any cause of death for the period of time 1962-75. Refinement of the data to municipalities and specific analyses to quantify biases in the approach will be pursued. (B) Case-control studies - The contractor will interview each selected cancer case and matched controls (or their families when appropriate) to ascertain occupational, environmental, and personal characteristics of the study population. These studies will be performed in areas which have been found to have exceptionally high mortality rates.

The sources of data are death certificates, interviews of living cases and controls, occupational and industrial information from interviews of next-of-kin, and Social Security data.

Major Findings: The contractor has concentrated on four main projects during the July 1, 1980 to June 30, 1981 contract year. These activities included preparation of an atlas of cancer mortality in New Jersey, field operations for a population-based retrospective case-control study of lung cancer, a retrospective case-control interview study of liver cancer, and preliminary analyses of data collected during retrospective case-control bladder cancer studies.

The contractor's work on the descriptive epidemiology of cancer mortality in New Jersey is in its final stages. The structure of the finished product has evolved into a two volume report which will be entitled the "Descriptive epidemiology of cancer mortality in New Jersey 1949-1967". The first volume presents summary information, descriptive analysis, discussion of the results of statistical analysis, and graphic displays of basic data. The second volume is a statistical appendix which presents a series of basic data tables for each analysis presented in the first volume.

The lung cancer study is in its data collection phase. Our current estimates of response rates are between 84-89% for cases and from 78-82% for controls. Several distinctive features of lung cancer mortality during the 1949-1976 period came to light as a result of the contractor's work of the descriptive epidemiology of cancer mortality in New Jersey. First, cancers of the trachea, bronchus, and lung are perhaps the single most important cause of cancer mortality in New Jersey. Second, lung cancer mortality rates for county populations in New Jersey consistently exceeded national lung cancer mortality rates throughout the 1950-1975 period. Third, and perhaps most important, geographic distribution of lung cancer mortality rates was distinctly non-uniform throughout New Jersey. Refinement of the geographic patterns of lung cancer mortality rates to the municipality level showed several areas within the State with distinct concentrations of significantly high lung cancer mortality rates for white males. The retrospective case-control study which is ongoing is examining these high risk areas to determine occupational and environmental factors responsible for this geographic pattern.

A retrospective case-control study was conducted to identify risk factors associated with cases of primary liver cancer in New Jersey. This study concentrated on the relationship of possible toxic exposures to the development of liver cancer. Particular emphasis was placed on the use of pesticides including those containing arsenic. Preliminary analyses of retrospective interview data show several variables to be associated with liver cancer in New Jersey. Of particular interest is the association of employment and the agricultural industry and liver cancer. Distinctions were made between employment as a farm owner, or manager, or a farm laborer. A significant risk was found only for male farm laborers who are likely to have contact with pesticides. Analysis of farm work by decade shows significant case-control differences, but no specific time period unique to our arsenicals was noted. A manuscript is currently in preparation which will present these findings.

Significance to Biomedical Research and the Program of the Institute: Recent reports of excessive mortality from bladder cancer in New Jersey need to be followed up by means of a project such as this to assess the relative contribution to this excess from industrial (occupational) as well as common environmental exposures. This type of investigation also fits in well with the specific interest in the geographic distribution of malignancy in the United States to which the Environmental Epidemiology Branch is committed.

Proposed Course: This contract covers projects in both the analytic and descriptive epidemiology of cancer. Analytic epidemiologic studies include: (1) the analysis of the data from case-control interview studies of bladder cancer, namely, the completion of the National Survey of Environment and Health, the completion of the study in high risk populations, and (2) a case-control interview study of lung cancer. Descriptive epidemiologic studies include the continuation of the development of the descriptive epidemiology of various cancer sites. It is proposed to investigate cancers of the stomach, pancreas, cervix, and ovary among others.

The work to date has pointed out several specific places in New Jersey which appear to warrant additional study. It is the intent of the contractor to concentrate on these areas to elicit additional etiologic information. The need to obtain occupation and industry information from living persons is recognized, and an approach has been set out. It is anticipated that the case-control investigations combined with the descriptive data compiled to date will point to specific industrial plants which should be investigated.

Date Contract Initiated: February 10, 1976

Current Annual Level: $500,000

PENNSYLVANIA UNIVERSITY OF (NO1-CP-91047)

Title: Special Projects in Hereditary Cutaneous Melanoma

Contractor's Project Director: Wallace H. Clark, Jr., M.D.

Project Officer (NCI): Mark H. Greene, M.D.

Objectives: To perform ultrastructural (electron microscopic) analysis of precursor nevi and related pigmented lesions. To develop educational materials for members of melanoma-prone families, the physicians involved in their care, and the pathologists responsible for interpreting pigmented lesion biopsies from high-risk family members. To perform light microscopic evaluation of the pigmented lesions removed from persons at high-risk of malignant melanoma.

Methods: Three separate educational program are to be produced using the clinical, photographic, and histologic material collected during our studies of melanoma-prone families. The production work has been subcontracted to E. J. Stewart, Inc. Selected pigmented lesions (including junctional nevi, compound nevi, lentiginous melanocytic dysplasia, epithelioid melanocytic dysplasia, halo nevi, spindle cell tumors, superficial spreading melanoma, and lentigo maligna melanoma) have been identified, fixec, and appropriately sectioned for electron microscopic study. Pigmented lesions removed from members of high-risk families will be collected and submitted to the contractor for standard evaluation.

Major Findings: All three educational videotapes have been completed and are now available for nationwide distribution. The electron microscopic studies are nearing their conclusion, with the major observation being that dysplastic melanocytes are virtually indistinguishable from malignant melanocytes at the ultrastructural level. In addition, the data suggest that lentiginous dysplasia may be the earliest stage of melanocytic abnormality with epithelioid dysplasia representing a more advanced abnormality that more closely resembles fully-developed melanoma. Fifty surgical biopsy specimens from 40 high-risk patients have been evaluated per our standard protocol.

Significance to Biomedical Research and the Program of the Institute
The dysplasia nevus syndrome has now been clearly identified as etiologically important in both hereditary and sporadic melanoma. The light microscopy studies have permitted our classification of members of high-risk families into subgroups of patients who are at either high risk or normal risk of melanoma. All of tese findings will contribute to the eventual control of mortality from this potentially lethal tumor.

Proposed Course: This study is now in its second and final year. A one-year extension was necessitated to complete the electron microscopy wori. It is anticipated that this contract will end in September 1981.

Date Contract Initiated: September 27, 1981

Current Annual Level: $98,000

TEXAS UNIVERSITY OF, MEDICAL BRANCH (NO1-CP-91037)

Title: Epidemiology of Primary Liver Cancer in Selected Counties of Texas

Contractor's Project Director: Patricia A. Buffler, Ph.D.

Project Officer (NCI): Thomas J. Mason, Ph.D.

Objectives: This project is designed to study mortality from primary liver cancer in selected counties in Texas. These counties were selected because they have the highest rates of primary liver cancer in this country.

Methods Employed: The contractor will use a case-control approach, selecting two controls for each case of primary liver cancer from the next deaths which occur that match on age (within the same five-year category), sex, and race. The occupation and industry of employment will be coded from death certificates, and, in addition, information regarding employment and exposure in specific industries will be ascertained.

Major Findings: A retrospective study of all liver cancer deaths in Brazoria, Orange, and Jefferson Counties, Texas, was conducted during the period 1960-1976. The purpose of this study was to evaluate the apparent excess mortality due to primary liver cancer (PLC) for residents of these counties and the possible association between PLC and occupational exposure, and to examine a possible association between PLC and environmental exposures.

One hundred seventy-six cases of liver cancer were identified among the residents of the three counties from a review of death certificates for the period 1960-1976. The age-adjusted liver cancer rates (ICD 155.0) were higher than the U.S. rates in 1960 for nonwhite males and females in Brazoria and for white and nonwhite males in Orange and Jefferson in 1950-1969. For the time period 1964-1975, liver cancer rates (ICD 155.0 and 197.8) age-adjusted to the 1970 U.S. population were higher than the U.S. rates for white and nonwhite females in Brazoria County, and for white males and females and nonwhite females in Orange and Jefferson counties. However, when all available hospital records and pathologic specimens were systematically reviewed, the number of confirmed cases observed was reduced to 54; i.e., to 30.6% of the total cases coded to liver cancer rubrics. Crude race-sex-specific rates of PLC in the study areas were similiar to the U.S. 1970 crude rates, though a deficit was observed in white males in Brazoria County and nonwhite males in Orange and Jefferson counties.

Occupational histories of the liver cancer cases were similar to those of the controls in Brazoria County, and significantly more controls than cases were employed in Orange and Jefferson counties. An environmental risk assessment revealed no difference between cases and controls with respect to residential patterns at time of death. "Nearness" scores to area industrial facilities, as estimated by a model using distance to plants weighted by their emissions, showed no difference between cases and controls. In addition, there was no interaction between employment status and environmental exposure for liver cancer cases and controls.

The results of this study are currently being summarized and several articles are in preparation. Specific emphasis will be given to a more detailed analysis of the work histories.

Significance to Biomedical Research and the Program of the Institute: This project will contribute to the NCI objective of accurately assessing the incidence and mortality of cancer and identifying factors related to cancer risk. An investigation of this type also fits in well with the specific interest in the geographic distribution of malignancy in the United States which the Environmental Epidemiology Branch has developed over the past several years. Reports of excessive mortality from primary liver cancer in Texas need to be followed up by means of a project such as this to assess the relative contribution to this excess from industrial (occupational) as well as common environmental exposures.

Proposed Course: This contract was extended for 3 months in order to code each job entry on the work history. This will permit more detailed analyses. These analyses will provide an assessment of the magnitude of an excess of liver cancer in these selected counties and also the association of this disease with potential occupational exposures in this part of the U.S.

Date Contract Initiated: April 26, 1977

Current Annual Level: $25,000

TEXAS UNIVERSITY OF, SYSTEM CANCER CENTER (NO1-CP-01051)

Title: Familial Cancer in Melanoma Patients

Contractor's Project Director: David Anderson, M.D.

Project Officer (NCI): Mark H. Greene, M.D.

Objectives: To define the incidence and types of cancer among first-degree relatives of patients with malignant melanoma in order to quantify the risk of melanoma among relatives and to identify other associated neoplasms. Pathology verification of melanoma cases and other pigmented lesions will be obtained through a subcontract with the University of Pennsylvania. Differences in risk of familial cancer will be assessed by melanoma subtype, and an effort will be made to determine the frequency of the dysplastic nevus syndrome in these melanoma patients.

Methods Employed: Family history of cancer will be obtained from the relatives of 433 consecutive melanoma patients treated at the M. D. Anderson Hospital during 1969-1970. The contractor will locate all relevant pigmented lesion biopsy material and transmit it to the subcontractor for pathology review. Clinical photographs of the melanoma patients will be reviewed to assess the frequency of the dysplastic nevus syndrome in these patients. Risk of melanoma and other cancers in first-degree relatives of cases will be assessed by comparing their frequency to that predicted by general population incidence rates. Analysis will be done by the Project Officer at NCI.

Major Findings: This project is less than six months old, thus, there are no findings to report.

Significance to Biomedical Research and the Program of the Institute: Having identified what is probably the single most important melanoma precursor to date, the dysplastic nevus syndrome, information on the frequency of this syndrome and melanoma risk among relatives of melanoma patients is now required. This information will permit more effective counselling and management of patients at high-risk of melanoma and will also be invaluable in planning melanoma prevention and education programs.

Proposed Course: This study was designed as a one-year project, during which time it is expected that all needed data will be collected. Data processing and analysis will be done by the Project Officer at NCI.

Date Contract Initiated: September 25, 1980

Current Annual Level: $95,852

TEXAS UNIVERSITY OF, MEDICAL BRANCH (NO1-CP-91025)

Title: Etiologic Study of Respiratory Cancer in Coastal Texas

Contractor's Project Director: Patricia A. Buffler, Ph.D.

Project Officers (NCI): Thomas J. Mason, Ph.D. and Linda W. Pickle, Ph.D.

Objectives: To examine the respiratory system cancer experience in selected areas of the State of Texas with specific emphasis on quantifying risk factors.

Methods Employed: The methodology is that of a case-control interview study. The contractor will interview each selected respiratory cancer case and matched controls (or their families when appropriate) to ascertain occupational, environmental, and personal characteristics of the study population. This study will be performed in areas which have been found to have exceptionally high mortality rates.

Major Findings: Analysis of the data began in May, 1981 and has not been completed for results to be available.

Significance to Biomedical Research and the Program of the Institute: Recent reports of excessive mortality from lung cancer in Texas pointed to the need for a project such as this to assess the relative contribution to this excess from industrial (occupational) as well as common environmental exposures. This type of investigation contributes to studies in the geographic distribution of malignancy in the United States to which the Environmental Epidemiology Branch is committed.

Proposed Course: To date several specific places in Texas have been pinpointed which appear to warrant additional study. The contractor will

concentrate on these areas to obtain occupational and industrial information from living persons of possible etiologic significance in respiratory cancer.

Date Contract Initiated: September 30, 1979

Current Annual Level: $165,000

VETERANS ADMINISTRATION (P00023)

Title: Mesothelioma and Employment: C. A Case-control Study Utilizing the Veterans Administration (VA).

Contractor's Project Director: Not available.

Project Officer: Robert Spirtas, Dr. P.H.

Objectives: To collect information to evaluate the role of occupational exposure in the origin of mesothelioma. Asbestos miners and insulators and shipyard workers are known to be at high risk for mesothelioma, but information on other industries where exposures to asbestos and other fibrous products are lower is incomplete. This study supplements the study using cases from tumor registries by providing additional cases from the VA.

Methods Employed: The project is a case-control design. Cases of mesothelioma from the VA and their matched controls will be interviewed (next-of-kin will be interviewed if the cases are deceased) to obtain work histories and other information pertinent to the origin of this tumor.

Major Findings: Not available.

Significance to Biomedical Research and Program of the Institute: This contract will provide data needed to estimate the risk of low-level asbestos exposures in a variety of industries and occupations. The rising incidence of this tumor and the widespread exposure to asbestos underscores the need to identify new exposure groups so that preventive action may be taken.

Proposed Course: Award contract in August, 1981.

Date Initiated: Not available.

Estimated Annual Level: $50,000

YALE UNIVERSITY - (N01-CP-01029)

Title: Risk of Cancer Following Multiple Chest Fluoroscopies for Tuberculosis in Connecticut.

Contractor's Project Director: Jennifer Kelsey, Ph.D.

Project Officer (NCI): John D. Boice, Jr., Sc.D.

Objectives: To determine the long-term health effects of multiple low-dose radiation exposures in men and women and to estimate the risk of radiation induced leukemia, lung cancer, and breast cancer.

Methods Employed: All patients discharged alive from major Connecticut State tuberculosis hospitals between 1930 and 1952 are being studied. Hospital records are being used to determine the extent of the tuberculosis and the number of fluoroscopic examinations performed on each patient. Death certificates will be obtained for those patients who have died. The Connecticut Tumor Registry will be used to determine the incidence of all cancers in this population.

Major Findings: A preliminary review of 148 charts was done to determine the quality of data concerning number of air refills received by pneumothorax patients. Seven physicians and one technician have been interviewed regarding the technique. A precoded abstract form has been developed. Criteria for inclusion of patients into the study have been established. Information on fluoroscopic equipment has been gathered.

Significance to Biomedical Research and the Program of the Institute: Persons repeatedly exposed to radiation over a period of years will be evaluated. This study will determine whether low-dose fractionated exposures are as effective in producing cancers as single high-dose exposures and, thus, has relevance to cancer etiology. This project is also a major component of the Institute's expanded program on the biological effects of ionizing radiation.

Proposed Course: Data collection will proceed for approximately two more years followed by analysis and publication of study findings.

Date Contract Initiated: September 30, 1980.

Current Annual Level: $198,663.

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CARCINOGENESIS EXTRAMURAL PROGRAM

ORGANIZATIONAL OVERVIEW

The Carcinogenesis Extramural Program (CEP): (1) develops, evaluates and administers the Division's program of extramural research in cancer causation and prevention; (2) is responsible for program management, including improved methods and practices; (3) maintains liaison between extramural activities and various organizations and scientists; and (4) assists in allocating resources and evaluating program priorities for these activities. To accomplish its goals, the program makes use of a variety of instuments which include traditional research grants, interagency agreements, and contracts.

To integrate the management of these diverse research activities and better coordinate the activities of the many investigators involved, the CEP was established. Technical review of all research proposals (contract and grant) is conducted by the Division of Extramural Affairs (DEA) utilizing traditional peer review groups whose members are drawn from the outside scientific community. Steps are now being taken to include the technical review of research support and resource contracts within the DEA structure. For contracts, review for relevance, priority and need are still performed by the senior staff of the Division of Cancer Cause and Prevention.

The Carcinogenesis Extramural Program contains three branches: Biological Carcinogenesis Branch, Chemical and Physical Carcinogenesis Branch, and Special Programs Branch. It has a current on-board staffing level of 37 full-time permanent positions and has a budget of $133 million in Fiscal Year 1981.

Significant changes in Fiscal Year 1981: We have continued to reduce the level of contract support for resources to the research community in general, and in addition, are continuing to investigate the feasibility of introducing cost recovery mechanisms into our resources activities. Such modifications are expected to further reduce the cost of providing resources in future years since we anticipate that some further reductions will be required. Careful planning is needed to avoid disrupting effects in the general research community.

We have continued our efforts to reduce or phase out contract support of investigator-initiated research in fields where grants seem to provide adequate coverage. Funds made available in this way will be used to stimulate the development of high priority areas of research which are inadequately covered by grants. An example of such a new initiative might be a Request for Grant Applications (RFA) to conduct research on the underlying basis and mechanism of promotion phenomenon observed in the carcinogenic process.

The overall effects of these modifications continue to be: (1) a gradual transfer of current resources to a cost reimbursement system; (2) increased availability of funding to support the development of new resource activities required by the changing needs of investigators; (3) the phasing out of contract-supported research in areas adequately covered by research grant applications; and (4) an increased use of RFAs to stimulate research activity in high priority areas.

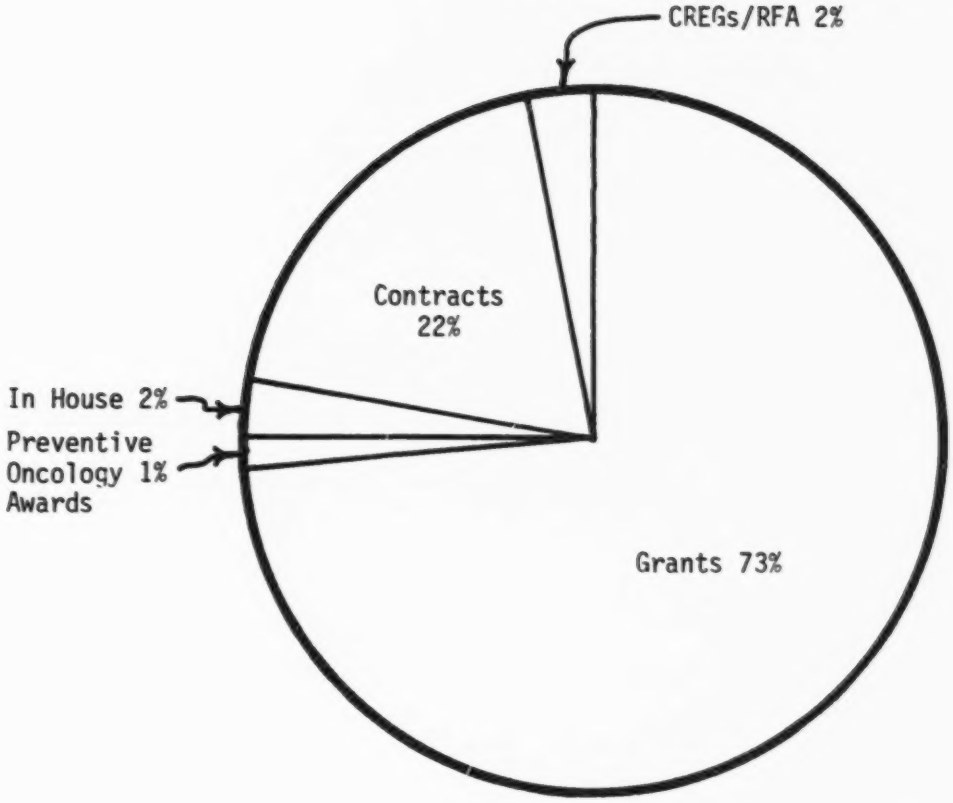
Considerable progress has been made in the past year toward meeting the cause and prevention related goals of the Carcinogenesis Extramural Program. Such accomplishments are well summarized in the reports of individual research areas included in the Branch summaries.

The Biological Carcinogenesis Branch research program continues to provide valuable insights into the mechanisms of viral carcinogenesis. It is now increasingly likely that viral information, present as nucleic acid sequences in the genes of apparently normal cells and replicating with them, is intimately involved in the development of some cancers. A major step toward understanding the mechanisms of viral transformation has occurred with the recent identification of the protein product of the avian sarcoma virus "src" gene. This is the first viral transforming gene product which has been found to be associated with an important enzymatic activity (protein kinase) that could potentially effect the large number of cellular alterations which occur upon cellular transformation.

The Chemical and Physical Carcinogenesis Branch reports considerable progress toward an understanding of the metabolism and pharmacokinetics of carcinogenic substances, e.g., polycyclic hydrocarbons, nitrates, arylamines. In the area of chemoprevention, progress continues to be made in the development of model systems for the investigation of the phenomenon, and increased emphasis is being placed on studies designed to elucidate mechanisms of action. The new initiative undertaken with regard to "Interspecies Comparisons in Carcinogenesis" (using the research grant mechanism) has had an excellent response and resulted in a number of awards.

Within the Special Programs Branch, activities centering on mathematical modeling of both the carcinogenic process and epidemiologic study design and analysis are well under way, as are the development of new or improved techniques for data analysis in a number of contexts (Biometry). The recently reported possible association of coffee consumption with elevated risk for pancreatic cancer in both sexes is of great research interest, particularly since the elevation in risk was not affected by controlling for cigarette smoking. In addition, the recent suggestion that Hodgkin's Disease in young adults may be a reaction to a common viral infection modified by late age at exposure has generated considerable interest and is likely to stimulate new work in this area.

FY '81



Carcinogenesis Extramural Program

($133.4 million)

	$	%
Contracts	29.3	22
Grants	98.8	73
CREGs/RFAs	3.2	2
Preventive Oncology Awards	.5	1
Sub-Total	131.8	98
In House	1.6	2
TOTAL	$133.4	100%

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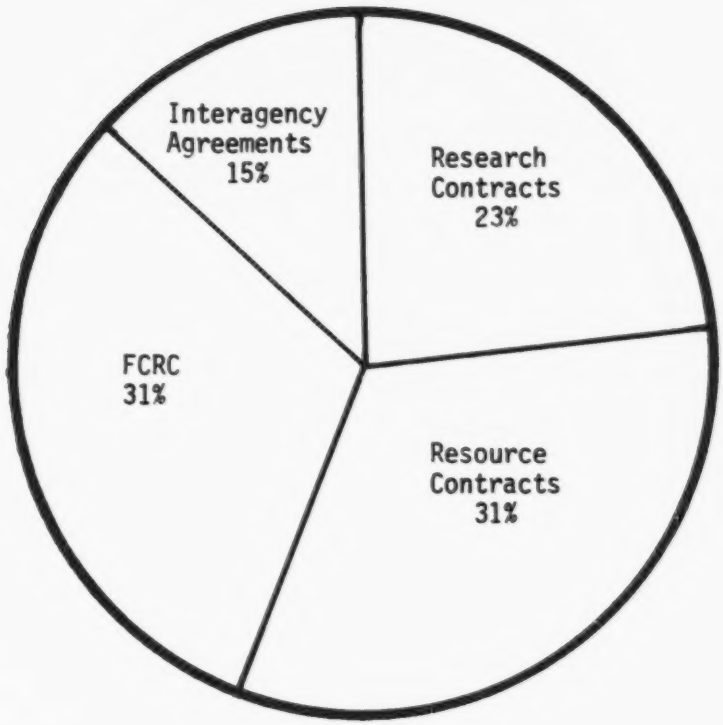

Distribution of Carcinogenesis Extramural Program Contract Funds

($29.3 million)

	$	%
Research Contracts	6.6	23
Resource Contracts	9.1	31
Interagency Agreements	4.3	15
Frederick Cancer Research Center	9.3	31
TOTAL	$29.3	100%

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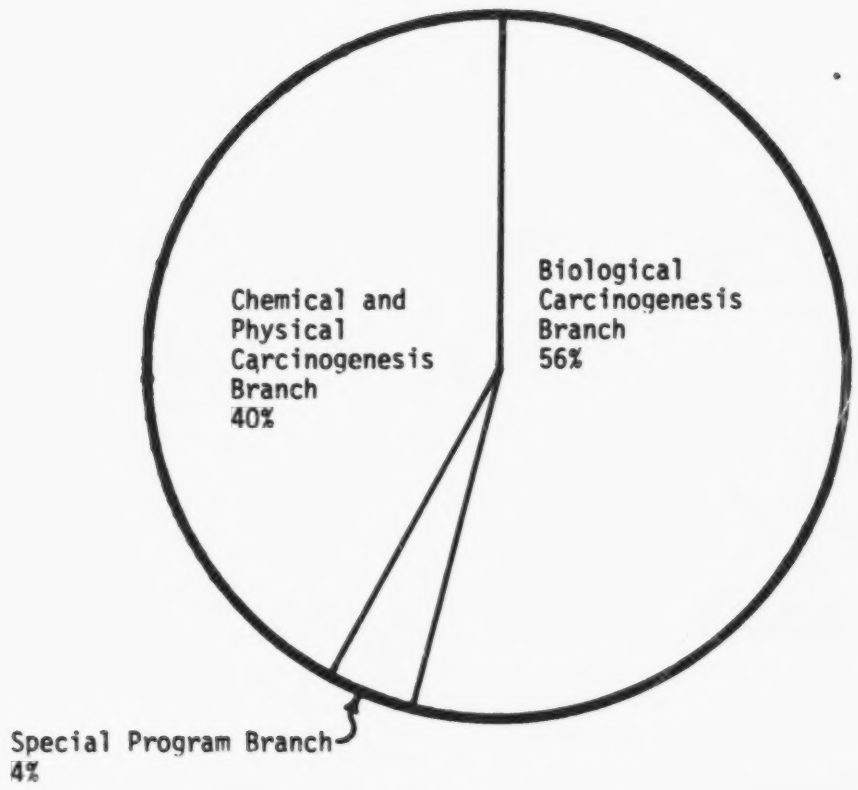

Carcinogenesis Extramural Program Contracts by Branch

($29.3 million)

	$	%
Biological Carcinogenesis Branch	16.4	56
Chemical and Physical Carcinogenesis Branch	12.1	40
Special Programs Branch	.8	4
TOTAL	$29.3	100%

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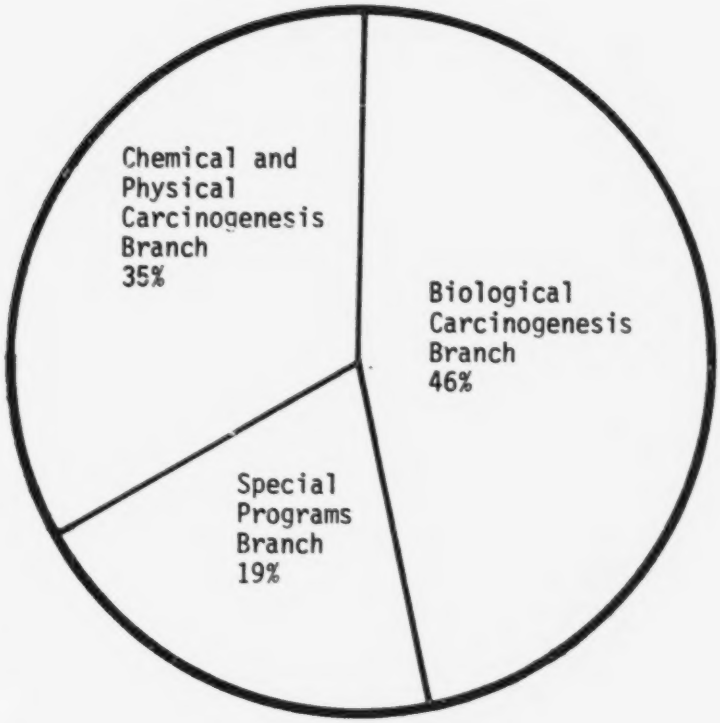

Carcinogenesis Extramural Program Grants by Branch

($102.5 million)

	$	%
Biological Carcinogenesis Branch	47.2	46
Chemical and Physical Carcinogenesis Branch	36.2	35
Special Programs Branch	19.1	19
TOTAL	$102.5	100%

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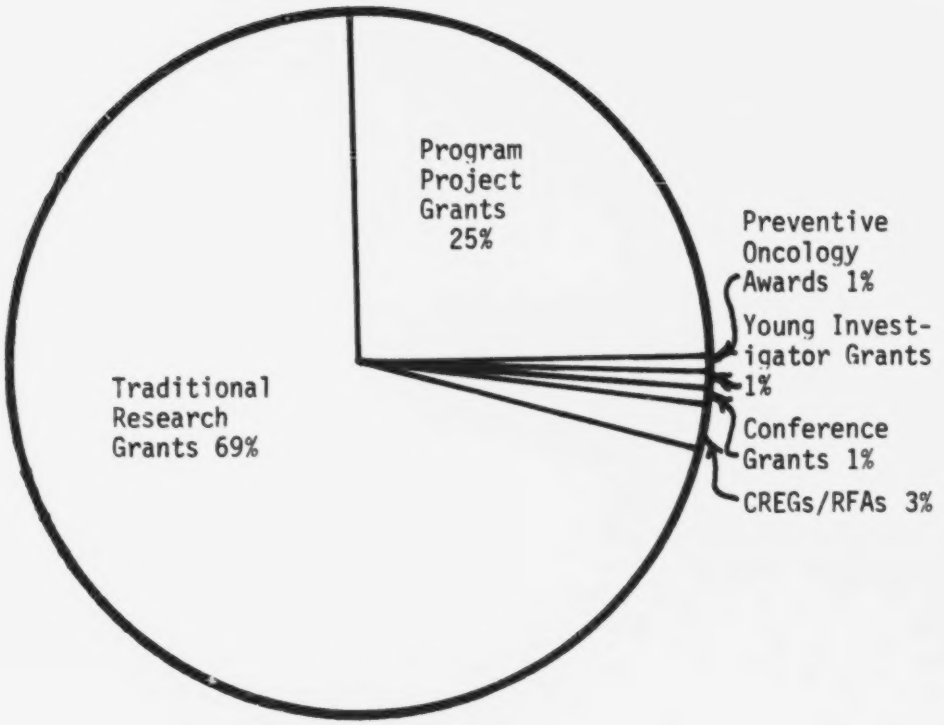

Distribution of Carcinogenesis Extramural Program Grant Funds

($102.5 million)

	$	%
Traditional Research Grants (R01s)	70.9	69
Program Project Grants (P01s)	26.8	25
Conference Grants (R13s)	.4	1
Young Investigator Grants (R23s)	.7	1
Preventive Oncology Awards (K07s)	.5	1
Cancer Research Emphasis Grants/RFAs (CREGs/RFAs)	3.2	3
TOTAL	$102.5	100%

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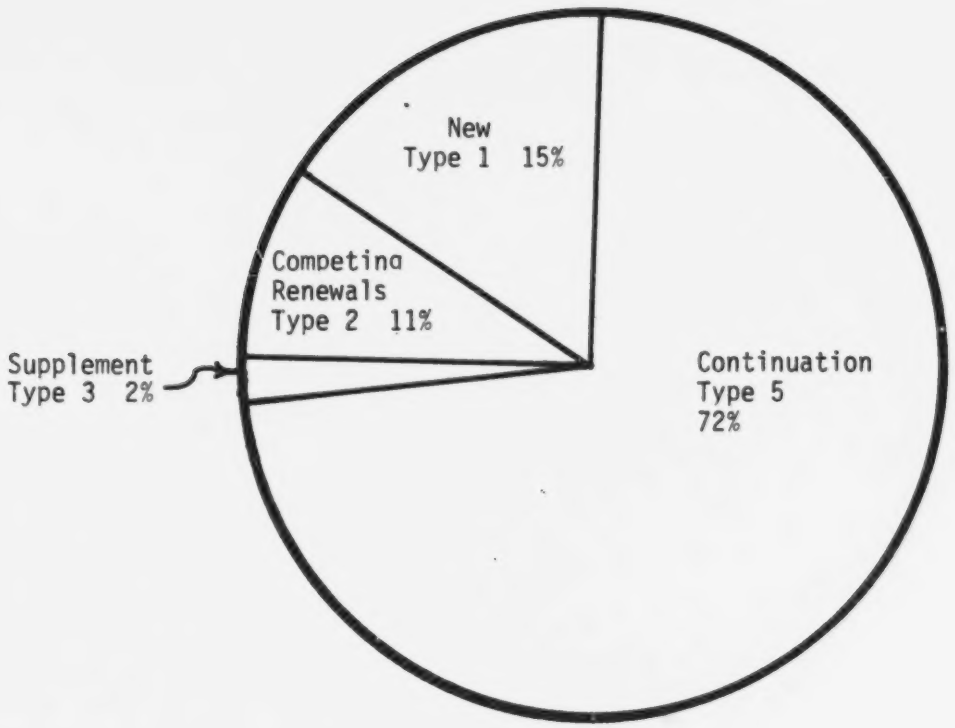

Carcinogenesis Extramural Program Grants by Type

($102.5 million)

	$	%
New (T1)	15.7	15
Competing Renewals (T2)	11.5	11
Supplement (T3)	1.6	2
Continuation (T5)-Including Preventive Oncology	73.7	72
TOTAL	$102.5	100%

BEST DOCUMENT AVAILABLE

SUMMARY REPORT

BIOLOGICAL CARCINOGENESIS BRANCH

The Biological Carcinogenesis Branch (BCB) plans, develops, directs and manages a national extramural program of basic and applied research concerned with the role of biological agents as possible etiological factors or co-factors in cancer and on the control of these agents and their diseases; establishes program priorities, and evaluates program effectiveness; provides a broad spectrum of information, advice and consultation to individual scientists and institutional science management officials relative to NIH and NCI funding and scientific review policies and procedures, preparation of grant applications and choice of funding instruments; provides NCI management with recommendations as to funding needs, priorities and strategies for the support of relevant research areas consistent with the current state of development of individual research activities and the promise of new initiatives; plans, develops and manages research resources necessary for the conduct of the coordinated research program; develops and maintains computerized data management systems; and plans, organizes and conducts meetings and workshops to further program objectives, and maintains contact with the relevant scientific community to identify and evaluate new research trends relating to its program responsibilities.

The extramural activities of the Branch are accomplished through contractual agreements with universities, research institutes, and commercial organizations, and through traditional individual research grants and program project grants with universities and research institutes. Currently, the Branch administers over 430 research activities with an annual budget of approximately 63 million dollars. The research projects of the branch divide into three main categories. Research programs on viruses with a DNA core which are known or suspected to be involved in the induction of malignant transformation are included in the DNA Virus Studies. Research dealing with RNA core viruses which are known or suspected of involvement in the malignant transformation of animal and human cells are covered by RNA Virus Studies components. The Branch program component designated RNA Virus Studies I involves research concerning murine, feline, bovine, primate, and hamster viruses. The program component designated RNA Virus Studies II incorporates research involving avian tumor viruses, pox viruses, myxoviruses, picornaviruses, hepatitis B virus, and plant viruses. Those research elements previously listed under Special Projects have been redistributed to DNA Virus Studies I and RNA Studies II. The name "Special Projects" is no longer being used.

To facilitate and support these research activities, the research resources component of the Branch is responsible for planning, developing, allocating and maintaining the biological research resources necessary for the extramural research effort. The BCB Research Resources Contract Review Group provides general support and recommendations concerning biological resources, and conducts appropriate reviews for the BCB research resources contracts. Research resources is assisted by a data management element which is responsible for the automated retrieval and inventories of BCB resources, computer-systems planning, and automated analysis and management support. The automated inventories include the research resources virus inventory, the serum collection, the human tissue collection, and the virus, antisera and cell culture collections of the satellite resources systems.

An additional responsibility of the Branch is the monitoring and evaluation of the virus and reagent production activities of the Viral Resources Laboratory at the NCI Frederick Cancer Research Center (FCRC). The day-to-day management of the distribution of the research materials prepared at FCRC for extramural use is the responsibility of the BCB research resources component.

Table I focuses on mechanisms of support of extramural research and related activities in the area of biological carcinogenesis. The total budget in FY 81 was approximately 5 million dollars less than the FY 80 budget. The primary reason for the decreased funding was the programmed termination of research contracts. In contrast, the research grants actually showed an increase of approximately 3 million dollars. Approximately 75 percent of the Branch budget is now allocated to the support of grant research. Table II provides an estimate of grant and contract support, respectively, in each of the four Branch components described above. The Branch administers 61 contracts and 369 grants.

Investigations carried out in the Biological Carcinogenesis research program during the past year have produced valuable insights into the mechanisms of viral carcinogenesis and suggested means by which the transformation of cells from the normal to the malignant state occurs and might be arrested. It is now increasingly likely that viral information, present as nucleic acid sequences in the genes of apparently normal human cells and replicating with them, is intimately involved with the development of cancer. Triggered by chemical carcinogens, radiation, hormones, the ageing process, and other influences, these viral sequences may direct the synthesis of the proteins responsible for malignant transformation of the cell. For this reason, increased attention has been given to studies defining the interaction of viruses and cells in both animal and human cancers and to identify minute regions of virus and cell chromosomes which are responsible for malignancy; to understand the molecular pathways of viral replication and to identify virus products which may trigger the transformation of a cell to malignancy; and to understand and enhance immune mechanisms which ultimately may prevent cancer.

Of the many oncogenic viruses currently under investigation, the avian sarcoma virus (ASV) represents one of the best model systems available for delineating the molecular mechanisms involved in viral-induced carcinogenesis. One of the four major genes of the ASV genome, termed src, is responsible for the induction and maintenance of the transformed cell state in vitro and sarcomas in vivo. Considerable progress towards a better understanding of these mechanisms has been made by the recent identification and characterization of the protein product of the ASV src gene - pp60src. This is the first viral transforming gene product which has been found to be associated with an important enzymatic activity (protein kinase) that could potentially effect the large number of alterations occurring upon cellular transformation. Analogous products are induced by other tumor viruses and in some instances have been identified. The importance of these findings is that they may lead to the formulation of general principles of the biogenesis of neoplasia and provide useful reagents for diagnosis, prevention or treatment of cancer.

One approach to the detection of virus information in human tissues is the construction of hybridization probes to detect homologous type C viral sequences. Immunologic evidence suggests that certain proteins produced by the Hodgkin's lymphoma SU-DHL-1 cell line are antigenically related to products coded for by viruses of the GaLV/SSAV groups; therefore, this group of viruses has been used

TABLE I
BIOLOGICAL CARCINOGENESIS BRANCH
(Extramural Activities - FY 1981 - Estimated)

	No. of Contracts/Grants	$ (Millions)
Research Contracts	41	3.50
Research Grants	357	46.60
Traditional Project Grants (320 grants; $31.23 million)		
Conference Grants (3 grants; $0.15 million)		
New Investigator Research Grants (13 grants; $0.29 million)		
Program Project Grants (21 grants; $14.93 million)		
Cancer Research Emphasis Grants	12	0.56
Research Resources Contracts	20	6.60
Frederick Cancer Research Center	2	5.30
Research Effort (1 contract; $4.80 million)		
Extramural Resources ($0.50 million)		
TOTAL	433	62.56

TABLE II
BIOLOGICAL CARCINOGENESIS BRANCH
(Contracts and Grants Active During FY 1981)

	FY 81 (Estimated)			
	CONTRACTS*		GRANTS**	
	No. of Contracts	$ (Millions)	No. of Grants	$ (Millions)
DNA Virus Studies	15	1.2	161	21.7
RNA Virus Studies I	26	2.3	115	13.4
RNA Virus Studies II	0	0	93	12.1
Biological Research Resources	20	6.6	0	0
TOTAL	61	10.1	369	47.2

* Does not include FCRC (2 contracts).
** Includes Cregs.

in most hybridization studies. In preparation for these studies the gibbon type C (GaLV) viral genome has been cloned in a lambda phage vector and mapped with restriction endonucleases. Several fragments have been subcloned in the pBR322 plasmid vector and labeled by nick-translation for use as a molecular hybridization probe. Further characterization of the probe is ongoing to clarify the possible associations.

The reactivity of a monoclonal antibody [(3D7)(2B9)] which detects a 28,000 dalton protein p28 in the SU-DHL-1 virus and also crossreacts with the p28 of SSV-1/SSAV has been compared with that of a heterologous rabbit antibody which has previously been shown to be monospecific for the p28 of SSV-1/SSAV. In competition radioimmunoassays, SSAV-1/SSAV preparations competed with the SU-DHL-1 p28 for binding to the [(3D7(2B9)] antibody, but with a different slope than that observed with the homologous antigen. This indicates that the SSV-1/SSAV antigenic site with which this antibody crossreacts is only partly homologous with the antigenic site on the SU-DHL-1 viral p28. Also, the monospecific heterologous antiserum to SSV-1/SSAV p28 precipitates two proteins in the 60-65,000 dalton range which are translated from mRNA of approximately 30-355 in SSV-1/SSAV infected cells, whereas the [(3D7)(2B9)] monoclonal antibody to the SU-DHL-1 viral p28 precipitates only the 65,000 dalton translation product of SSV-1/22AV viral RNA.

Using a method for demonstrating primer tRNA binding to poly(A)RNA molecules, it is now possible to demonstrate various specific poly(A)RNA molecules in cultured human cells. Initial studies indicated that a human breast cancer cell line (ALAB) contained a unique primer tRNA binding an RNA molecule of 24S size. This molecule is present in all human breast cancer epithelial cell lines examined, but not in epithelial cell lines of other human cancers, nor in the fibroblast cells derived from human breast cancers. It apparently binds selective tRNA species, the identity of which is being investigated. In the reverse transcription reaction in which avian myeloblastosis reverse transcriptase is added to a mixture of poly A RNA preparations of the cell and total tRNA's, a copy of DNA of about 140 base lengths linked to a 70 base RNA primer can be demonstrated. Preliminary results suggest that content of this RNA molecule in the ALAB cells may be stimulated by hydrocortisone treatment.

Studies are continuing to define an antigen in human mammary neoplasia which is immunologically-related to the major envelope glycoprotein (gp52) of murine mammary tumor virus and to determine the significance of this crossreactivity. Significant progress has been made in the purification of the gp52 related antigen from clones 8, 10, and 11 of the 47D human breast tumor cell line. The particles containing the relevant antigen banded at 1.16 to 1.19 g/ml and the yield was 0.2 - 0.5 mg of particles/liter of medium. Certain characteristics of these particles have been established, including incorporation of radioactive uridine, leucine and glucosamine. The uridine incorporated material can be extracted by phenolization as an RNA molecule which bands at 1.65 in a cesium sulfate density gradient and sediments as a 70S molecule in an SDS sucrose gradient. It further appears that the yield of the 70S RNA with clones 10 and 11 increases as the estradiol concentration is increased from 10^{-10} M to 10^{-6} M. Appropriate examination of the particles shows the presence of reverse transcriptase in all particle preparations from the three clones examined. An endogenous reaction with radioactive deoxytriphosphates leads to incorporation into an acid-insoluble DNA which bands at a density of 1.45 in cesium sulfate gradient. However, if the total nucleic acid is run, it is found that a portion of the DNA sediments with the 70S RNA molecule and the DNA is then found in the RNA density

region of a cesium sulfate density gradient. Pretreatment with either alkali or ribonuclease shifts these counts to the DNA region of the density gradient. These results further support the presence of a viral-like RNA core and reverse transcriptase.

Although clones 8, 10 and 11 of the tumor cell line appeared to possess characteristics suitable for the production of human antigen, it was noted that the antigenic activity of the clone 10 particles split into two peaks in glucose gradients, one banding at 1.16 to 1.19 g/ml and another between 1.20 and 1.24 g/ml. The latter is usually the density region in which cores of C and B type particles are found, and it seemed possible that this might be the case here. After extensive testing in glucose gradients, it was found that all of the antigen crossreacting with the human gp52 from all three cell line clones shifted in a similar way to the 1.20 - 1.24 g/ml region.

Studies using purified viral structural proteins and the corresponding antisera for immunological control of viral disease have been performed in mice. AKR mice were treated with heterologous anti-gp71 antibodies under various conditions in order to establish the optimal criteria for effective suppression of leukemia development. The strongest effect was observed when mice were treated at birth; and when this regimen was used, prior treatment of the mothers did not provide additional protection. If treatment was delayed until day 3, the beneficial effect of the serum diminished sharply, emphasizing the presence of a narrow window very early in the life of the AKR mouse when antibody must be present in order to have an effect on subsequent leukemia development. A number of parameters were examined in the experimental mice and as in a previous study, suppression of leukemia, which occurred in 68% of the animals, correlated with the elimination of viremia and appearance of natural anti-viral antibodies. Interestingly, the results suggest that antibody therapy is primarily effective against the thymic form of the disease.

A 55,000 dalton rat cell membrane glycoprotein (gp55), possibly related to the transformation process, has been purified to homogeneity and characterized. This protein was originally identified in preparations of a defective pseudotype of the Kirsten sarcoma virus and shown to be present in several rodent retrovirus particles. The gp55 was purified from this defective virus by conconavalin A and heparin affinity chromotography as well as by preparative SDS-gel electrophoresis. The ^{125}I-labeled gp55 was precipitated by antisera against rodent retroviruses, but not by monospecific antisera against purified type C virus structural proteins, thus indicating that gp55 was retrovirus associated, but unrelated to known retrovirus structural proteins. Competition radioimmunoassay indicated that the gp55 is a cell membrane glycoprotein associated in high concentration with retroviruses.

At the present time, breast cancer is known to be caused by a virus in only one animal species, the mouse. The mouse mammary tumor virus (MuMTV) mouse model system has been utilized for a variety of studies, including MuMTV replication and expression, MuMTV biology and characterization, and the effect of viral-chemical interaction on tumor induction. The expression and function of each MuMTV is influenced by many factors (genetic, hormonal, environmental) operating within and on each mouse. The neoplastic progression of the murine mammary gland involves an intermediary stage, the hyperplastic alveolar nodule (HAN), which can be morphologically visualized as lobular alveolar tissue in a non-pregnant, non-lactating host. The HAN can be surgically removed and transplanted into the

cleared fat pad of isologous hosts. The resultant growth of the HAN is the hyperplastic outgrowth (HG), which is delineated by the boundaries of the fat pad, and which has a higher tumor risk than normal mammary epithelium. This transplantation technique allows investigators to experimentally manipulate the HAN and to amplify the number of cells in the HAN for biochemical studies. By serially transplanting the preneoplastic HAN in mammary fat pads, HG have been established in strains C3H, GR, BALB/cNIV, and BALB/cC3H. New outgrowth lines are in the process of being developed from three low tumor incidence mouse strains [C3H/StWi, (C57B1 x DBAf)f_1, and BALB/cVo]. Six different HAN lines have been established from DMBA-treated or pituitary-isograft-bearing BALB/cVo animals, which express the putative endogenous MuMTV. All the lines are tumorigenic. Preliminary results indicate the outgrowth lines can grow and are tumorigenic in BALB/c animals, as well as in the parental BALB/cVo animals. All the HAN lines and most of the tumors which arose from the HAN lines expressed MuMTV antigens. Restriction maps of the five HGS derived from BALB/cfC3H strain demonstrate that all contain the 2.5×10^6 dalton Pst I fragment indicative of the exogenous C3H MuMTV. All HGs have their own unique restriction patterns and these patterns appear to be stable during the fourth and eighth through eleventh transplant generations. Some tumors which arise from these HGs contain additional restriction bands. However, all tumors contain the restriction pattern observed in their respective outgrowths.

In continuing studies of the association of DNA viruses with human malignancy, the Epstein-Barr virus directed nuclear antigen (EBNA) has been prepared from Raji cells to a purity of 90-95 percent. Peptide mapping and cleavage studies have shown that the antigenically active 48K component, EBNA, has no peptides in common with the cellular proteins that co-purify with it in the early phases of purification nor with degradation products of the transformation-related 53K protein. The murine 53K protein present in SV40-transformed and in chemically induced mouse sarcoma cells is closely related to the human 53K protein. The murine 53K protein present in SV40-transformed and in chemically induced mouse sarcoma cells is closely related to the human 53K component prepared from Raji cells. The host coded 53K protein is associated with the 48K DNA binding protein and is structurally similar since monoclonal antibodies to the 53K protein also bind to the 48K protein. Since discrimination between the two components is much better in human sera, human x human hybridomas are being established in order to aid in defining the association between these antigens and their roles in the transformation process.

In a study of very late relapses in Burkitt's lymphona (BL) patients, it was observed that 10 percent of the patients may relapse after remission periods of one to six years (the duration of study). These patients maintained elevated early antigen-restricted type (EA-R) antibody titers throughout their remission period, indicating that they were not out of danger as was subsequently confirmed by the later relapses. Whether the relapses are due to surviving BL cells or to new tumor induction is not known. In the absence of detectable tumors, the elevated EA-R titers may be attributed to a highly active persistent EBV infection.

In a collaborative multicenter study of EBV serological markers for diagnosis and prognosis in patients with NPC, preliminary information indicates that antibody titers to EBV-induced membrane antigen (MA) measured by the antibody-dependent cellular cytotoxicity (ADCC) assay has probable prognostic value. The data show that high anti-MA ADCC titers at diagnosis were indicators of a good prognosis following therapy whereas low titers were indicators of poor prognosis. The results also suggested that antibody to MA may function actively in vivo against

these tumors. Since earlier studies had shown an inverse relationship between IgA antibody titers to VCA and ADCC titers, it was thought that the prognostic significance of ADCC titers in NPC patients may be related to the presence of IgA antibodies in the sera of these patients. Studies were undertaken to determine the role of IgA antibodies directed against EBV antigens in ADCC. It was found that IgA antibodies could not mediate ADCC against EBV-infected target cells; all ADCC activity resided in the IgG fraction. However, IgA antibodies were able to block the ADCC reaction mediated by IgG antibodies. This was shown by both pretreatment and direct competition experiments, and indicate that low ADCC titers in patients with NPC could be caused by the blocking of IgG-mediated ADCC by IgA antibodies. This suggests that high levels of IgA antibodies in the sera of NPC patients may be detrimental to the patient because of the blocking activity, if the assumption is made that ADCC functions in vivo against this tumor. It will be important now to determine whether the blocking activity can be overcome with sera containing high titers of ADCC activity (IgG). If this is the case, then serotherapy may be of some benefit to NPC patients whose sera contain high levels of IgA antibodies and have low ADCC titers.

During the past year the Research Resources component has provided research materials and other supporting activities through contract operations representing four general areas. These include: activities directed toward production, characterization and distribution of purified viruses, viral reagents and appropriate antisera; activities concerned with animal resources, including production of pathogen-free species of animals, breeding of cotton-topped marmosets, maintenance of animal colonies including primates, and containment-type primate holding facilities; activities directed toward the production of specialized testing services for the examination of experimental materials; and activities concerned with acquisition, collection, storage, inventory and distribution of normal and malignant human specimens. Virus production and antisera preparation efforts were shared by a total of six contracts whose funding represented 49% of the total Resources budget. The animal resources area accounted for 23% of the budget, provision of testing and service efforts accounted for 26% of the budget, and human specimen acquisition and distribution accounted for the remaining 2%.

At the request of the Director, NCI, Research Resources has performed user surveys on a number of resource contracts. In these surveys, recipients of the goods or services provided by a number of resource contracts were queried by letter and their evaluations of the products or services they received, as well as their estimates of the future needs for these products or services, were sought. The results of these user surveys were forwarded to the Director of the National Cancer Institute. Based on the formal user surveys, as well as on "informal surveys" (conducted by telephone) and on the patterns of requests for various materials, several resource efforts are being cut back or eliminated. Another significant factor associated with the operation of the Research Resources program in this reporting period is the initiation of the system whereby the recipients of resource materials pay for the resources which they receive. Under this system, recipients of resources will reimburse the contractor for the costs of the materials and for the shipping costs. The contractor in turn will credit these proceeds against the monthly vouchers which he submits to the Government for payment under the contract. The Government then functions as a guarantor of a certain level of business or distribution activity rather than as the ultimate consumer of the resources distributed. This system, called the "payback" system, was initiated on May 19, 1981, with the inception of a new

contract for the production and distribution of avian myeloblastosis virus and AMV reverse transcriptase enzyme, and will be applied to other virus production and animal resource contracts as circumstances warrant.

SUMMARY REPORT

GRANTS ACTIVITIES

The Biological Carcinogenesis research program consists of the study of biological agents as possible etiologic factors or co-factors in cancer and on the control of these agents and their associated diseases. Emphasis is placed on viruses, viral products, and related cellular substances as tumor-inducing agents, and includes biological, biochemical, immunological, and physical investigations of actual, potential or suspected, oncogenic viruses, and their interactions with, and effects on their hosts at all levels of biological organization. In general, the emphasis of the program is on the dynamic relationship of biological agents to the oncogenic and oncologic process and applications arising from these considerations. The program may also include research on infectious processes in a host organism induced by oncogenic and possibly oncogenic viruses, since viral latency and persistence may impact on the development of malignancy. Research on gene expression and other cell regulatory functions, utilizing biological agents as tools, is also appropriate for the program, provided the relationship and significance to neoplasia is clearly indicated and documented. While the greatest interest resides in animal viruses and animal systems, including human, projects which involve other forms of life may also be considered if these offer unique advantages and their pertinence to neoplasia is documented. Within the Branch, the grants research activities are grouped into three components comprising DNA virus studies, RNA virus studies I, and RNA virus studies II.

In the DNA virus studies component there are approximately 155 grants. Of these, the major research emphasis lies in mechanisms of transformation which includes genome structure, function, and expression (76 grants); and virus-cell interactions (58 grants). In terms of the viruses being studied, 46 grants concern the herpesviruses (herpes simplex virus, 20 grants; Epstein-Barr virus, 15 grants), and 109 concern the better known smaller DNA viruses, the adenoviruses and papovaviruses.

Although human tumor cell lines in vitro are more remarkable for their diversity than their uniformity, they may share some common feature related to their transformed state. Human cell lines, whether derived from spontaneous tumors or transformed in vitro with simian virus 40 (SV40), were found to contain a 53,000 dalton phosphoprotein (pp53) in contrast to normal human cells in which this protein was not detected. Isoelectric focusing showed that pp53 comprised several species in both SV40-transformed and tumor cells. Comparison of the pp53 species from various cell lines showed they were similar but not identical. There was no evidence of SV40 involvement in any of the tumor cell lines, and it seems likely that the situation is analagous to that found in transformed mouse cells in which pp53 appeared in cells transformed by a variety of agents. Actively growing cultures of several types of normal human cells, skin fibroblasts, milk epithelial cells, showed no sign of pp53. It is therefore suggested that the presence of pp53 may be associated with at least some types of transformation. Other studies have shown that normal kidney epithelium and fetal brain cells,

which express high pp53 levels during exponential growth, show a prompt decrease in pp53 associated with contact inhibition of cell division. Malignant cells, on the other hand, continue to express pp53 after confluency and subsequent overgrowth of the monolayers. These results suggest that pp53 may also be involved in normal regulation of cell division and that malignant transformation leads to abnormalities in the control of pp53 expression (Crawford et al., 1981).

Cell transformation by polyoma virus appears to result from the activity of three viral early proteins, two of which (small and middle T antigens) are likely to be required for the expression of transformation, while large T antigen (the A gene product) probably promotes only its establishment. The role of large T antigen in rat cells transfected with temperature sensitive polyoma DNA or with purified fragments of wild-type polyoma DNA, some of which could not code for intact large T antigen, was studied. Transformation efficiency was 20-fold higher in the presence of an active A gene product than in its absence. Fully transformed colonies which could be obtained in the absence of an active large T antigen were tumorigenic in syngeneic animals. Restriction enzyme analysis of viral DNA sequences integrated into the genome of the transformed cell lines showed a strong correlation between the presence of an active viral A gene product and integration in a head-to-tail tandem arrangement of viral DNA molecules. In the absence of functional large T antigen, transformants were found to contain multiple nontandem insertions of viral DNA segments shorter than the infecting polyoma molecules. The results indicated that polyoma sequences can integrate into the host genome in tandem and nontandem arrangements and that the tandem mode of integration increases the efficiency of transformation promoted and controlled by large T antigen (Della Valle et al., 1981).

A total of 19 host-range transformation (hr-t) mutants of polyoma comprise a single complementation group. These mutants have become very valuable for studying and understanding the cellular changes related to transformation. The series of middle T antigens (63K, 56K, and 36K) and small T antigen (22K) are affected by the hr-t mutants. Of these, the 56K plasma-membrane-associated middle T antigen and the 22K small T antigen appear to be encoded, in part, directly by the hr-t region of the viral DNA. The 63K and 36K forms of T antigen appear only when a functional hr-t gene is present. Whether these are primary translation products or cleavage products of the 100K or 56K proteins is not yet clear. The minimal changes in the hr-t DNA identified so far which result in total loss of transforming ability of the virus consists of the addition of three base pairs followed by a base substitutuion. The interpretation that the A gene function is one of initiation and hr-t is one of maintenance agrees with the observation that middle T and small T antigens continue to be expressed in transformed cells, whereas the large T antigen is frequently missing (Benjamin et al., 1980).

Relatively little is known about the properties of a protein covalently bound (CBP) to the 5' termini of Adenovirus 2 (Ad2) DNA. Because of this linkage, it has been thought that CBP functions in viral DNA replication and thus could be a product of an early viral gene or of a

host-cell gene. Several Ad2 early polypeptides have been identified that are approximately the same molecular weight as CBP. These include polypeptides coded by early region E1A, E1B, and E2. Recent studies have shown that adenoviral serotypes in each of the five groups of human adenoviruses have CBPs with an apparent molecular weight of 55,000 firmly associated with their termini. In addition, it was shown that these CBPs are highly related in sequence and do not correspond to any of the known virus-coded early polypeptides. The CBPs also do not correspond to any virion protein labeled during late stages of infection. The remarkable similarity in the peptide maps of the five groups of CBPs suggests that, if CBP is coded by a viral gene, it must be a highly conserved gene. Since considerable heterology of virion proteins occurs among the different serotypes, CBP may interact with cellular proteins involved in DNA replication. This may be a further indication of a highly conserved viral gene for CBP. The DNAs of the adenoviruses in each of the five groups do show five to 20 per cent homology and thus CBP may be coded in those portions of the genomes that are homologous. The data, however, do not yet exclude the possibility that the CBP is coded by a cellular gene (Green et al., 1980).

The Epstein-Barr virus (EBV) is the causative agent of infectious mononucleosis (IM) and has a unique association with Burkitt's lymphoma (BL), a monoclonal B lymphocytic malignancy. EBV is not frequently associated with other B cell malignancies which occur in latently infected humans outside the BL endemic regions. One hypothesis which could explain this is that EBV from endemic BL regions differs from the virus elsewhere in its oncogenic potiential. An approach toward resolving this issue is the comparison of the DNAs of the viral isolates. The DNA of the B95-8 virus, an IM isolate, has been compared with the DNAs of W91, P3HR-1, and AG876, all BL isolates. Studies have shown that B95-8 DNA is largely colinear with the other three virus DNAs but has an Eco R1-C fragment which is $9X10^6$ daltons smaller. The additional DNA from the BL isolates is viral and not cellular with no detectable homology to human lymphocyte DNA. EBV DNAs from cultures of three IM-derived cell lines were found to have the additional sequences characteristic of the BL isolates. Thus the data suggests that B95-8 virus is an unusual deletion derivative which may have arisen as a result of laboratory passage. The function of the additional viral DNA located at the left end of the long unique region is not known, but it is not necessary for cellular growth, transformation, expression of any of the known EBV specific antigens, viral replication in vitro, or tumor induction in experimental animals. There is speculation that the additional DNA has selective advantage in that, in its natural host, EBV may be subjected to metabolic, hormonal, immunologic, or tissue effects which require expression of the additional DNA (Raab-Traub et al., 1980).

Studies on the mechanism of insertion of herpes simplex virus (HSV) DNA sequences into eukaryotic cells and on the expression of these genes have revealed several levels of virus-cell interactions. Continued progress in this research should permit the design of antiviral agents to inhibit selectively viral functions, and the analysis of HSV malignant transformation. During the past year, it was shown that the HSV thymidine kinase (TK) gene can be used as a marker in co-transformation experiments to identify cells that have been transformed with unlinked cloned genes. With this method,

sequences for the rabbit β-globin gene were introduced into mouse cells, providing a basis for studying globin gene expression. The TK gene was also introduced into mouse teratocarcinoma cells which were then reinjected into mice and formed solid tumors. In seven out of nine tumors examined the viral sequences remained in the same configuration that they had in the initial transformed cells. In addition, the TK gene was expressed in the absence of selective pressure.

In studies to gain an understanding of HSV latency and pathogenesis initiated by HSV, an important observation was made that HSV is able to induce both cellular and viral DNA repair shortly after infection of a variety of cell species, including human embryonic fibroblasts. Cellular repair was detectable three hours after infection with genital HSV (HSV-2) and continued through 14 hours when some repair and semiconservative replication of virus DNA was also noted. Almost all DNA synthesis between 20 and 24 hours post-infection was derived from repair synthesis of both cellular and viral DNA. The data suggest that HSV-2 induces repairable damage in cellular DNA without viral DNA replication and that the viral DNA accumulating in the infected cells has a structure that does not require incision for repair. It is of interest that study of another herpesvirus, human cytomegalovirus (HCMV), showed that HCMV infection did not induce repairable lesions in cellular DNA and also did not significantly inhibit host cell DNA synthesis. The observation that HCMV can induce HSV from a latent state in human cells was further described. The HCMV function for HSV stimulation appears to be an early HCMV event. As early as 12 hours after HCMV superinfection HSV RNA is expressed in latently infected cells, and within 24 hours after HCMV infection, infectious HSV is produced by reactivated cultures although HCMV is not yet synthesized.

In the RNA virus studies I component there are approximately 112 grants utilizing the murine (95), feline (9), primate (3), bovine (2), rat (2) and hamster (1) model systems. Of these, 55% are involved with studies of gene organization, control and expression; 34% are devoted to studies of virus-cell interaction; 8% support studies on detection in human material of activities or components characteristic of RNA viruses; 2% support research on cocarcinogenesis, and 1% involves a study on the inhibition of viral replication and cell transformation.

The murine retroviruses are a class of positive strand RNA viruses which cause a variety of tumors in vivo. They fall into two broad groups: the replication-competent leukemia viruses; and the replication-defective transforming viruses. Studies of the genomic structure, life cycle, and protein products of both of these classes of viruses have yielded a great deal of information about gene expression in general. Some researchers are convinced that the study of these two groups of viruses will soon tell us more about normal cellular processes and how they are disrupted in virally transformed cells. Most of the grants in the murine studies utilize viruses representing each group or the recombinants therefrom.

Studies by Goff, Gilboa, Witte and Baltimore, 1981, with cloned viral DNA, have elucidated the structure of the Abelson murine leukemia virus (A-MuLV) genome and the homologous cellular gene. Circular double stranded DNA produced after infection of mouse cells with A-MuLV was isolated and cloned

in the phage vector Charon 21A. The resulting clones of the A-MuLV genome show homology to the ends of Moloney MuLV and to a 3.5 kb central region containing sequences unique to Abelson virus. A 2.3 kb restriction fragment containing only A-MuLV-specific sequences was subcloned in the plasmid vector pBR322 and used as a probe for the cellular gene that had been acquired by the virus. DNA from all inbred mouse lines examined contains an identical region of homology spread over 11 to 20 kb. The cellular gene contains intervening sequences which are lacking in the viral genome. Rat, chinese hamster, rabbit, chicken and human DNA also show homology to the viral probe.

Abelson murine leukemia virus (A-MuLV) appears to encode only one protein, of 120,000 daltons, p120, which is a hybrid protein encoded in part by a portion of the A-MuLV genome derived from the gag gene of M-MuLV and in part by acquired cellular genetic material. It is possible that the p120 is involved in transformation. To examine the possibility that p120 modifies cellular protein through phosphorylation of tyrosine in vivo, Sefton, Hunter, and Raschke, 1981, have measured the abundancy of phosphotyrosine in protein in cells transformed by A-MuLV. Both lymphocytes and fibroblasts that have been transformed by A-MuLV contain 6- to 12-fold increased levels of the rare modified amino acid phosphotyrosine in their proteins. This observation, coupled with the fact that the p120 protein has been shown to undergo an apparent autophosphorylation to yield phosphotyrosine in vitro, suggests that A-MuLV encodes a protein kinase that phosphorylates tyrosine in transformed cells. These results are similar to those obtained previously with Rous sarcoma virus and suggest, by analogy, that the modification of cellular polypeptides through the phosphorylation of tyrosine may be involved in cellular transformation by Abelson virus. The p120 isolated from transformed cells contains phosphoserine, phosphothreonine and phosphotyrosine. The phosphotyrosine is found at two sites in the protein. Therefore the p120 may be a protein kinase that undergoes autophosphorylation in vivo.

DNA transfection is different from virion infection in that it bypasses all biochemical steps required for the synthesis of active viral DNA in the replication cycle of retroviruses. Transfection of cells with retrovirus DNA can lead to successful establishment of viral genomes via two routes. Either the transfected donor DNA is integrated directly into DNA within the recipient cells, or the donor DNA can serve, even while not integrated, as a template for the synthesis of progeny virus particles which are then able to spread horizontally through the transfected culture. In the case of Harvey sarcoma virus (HarSV) replication defectiveness precludes this spread. Goldfarb and Weinberg, 1981, showed that NIH 3T3 cells transfected with HarSV DNA may acquire deleted proviruses. Such proviruses lack the right end of the wild-type HarSV DNA genome corresponding to the 3'-proximal portion of the viral RNA. In subsequent genetic recombination studies utilizing deleted HarSV (delHarSV) proviruses Goldfarb and Weinberg, 1981, suggested a model to explain how leukemia viruses can recombine with cellular sequences to generate novel defective viruses. This model includes a two step process. The first step is the coincidental integration of an MLV genome adjacent to a cellular gene whose expression leads to a transformed

phenotype. Because of occasional errors in transcriptional regulation or lesions in the provirus, transcripts arise which are initiated within the viral genome and continue into the adjacent cellular sequence. For example, such transcripts might arise by failure to terminate at the right end of the provirus or by utilization of the right terminal redundancy as a transcriptional promoter. The consequences of such cotranscription would be an RNA molecule whose 5'-proximal sequences would be of MLV origin and whose remaining sequences would be of cellular origin.

Many such hybrid molecules would be structurally similar to the transcripts derived from the delHarSV proviruses used in these studies. They would then be able to participate in the second event, namely recombination with a competent MLV helper genome which exists in the same infected cell and with which these genomes share 5'-proximal homologies. This latter step occurs with surprisingly high frequency in the delHarSV-transformed cells studied here and might rapidly create a transmissible transforming virus once an integration event has generated the proper juxtaposition of viral and host cell sequences.

Van Beveren, et al., 1981, utilizing Moloney mouse sarcoma virus (Mo-MSV) which is able to transform fibroblasts in vitro and induce neoplasia in vivo, but is unable to replicate, have determined the complete nucleotide sequence of the transforming gene of Mo-MSV. It codes for a protein of 374 amino acids. The nucleotide sequence of the junctions between a murine leukemia virus and cellular sequences leading to the formation of the viral transforming gene have also been elucidated. The viral transforming sequence and its cellular homologue share an uninterrupted stretch of 1159 nucleotides, with few base substitutions. The predicted amino acid sequence of the mouse sarcoma virus transforming gene was found to share considerable homology with the proposed amino acid sequence of the avian sarcoma virus oncogene (src) product.

In addition to studies concerned with virologically transformed cells, DNA-mediated gene transfer has recently been applied to the study of chemically induced tumors. A series of chemically transformed mouse fibroblast cells have been extensively characterized. The DNAs of four of the 3-methylcholanthrene (3-MC) transformed mouse cells have been shown to possess biological activity and to induce transformation in recipient cells after transfection. Each of the four DNAs has been treated with one of six restriction endonucleases prior to transfection. Three of these enzymes destroyed the biological activity of all four of the DNAs. Since this pattern could arise by chance only with a probability of one in 10^{-5}, it was concluded that the transforming gene in the four independently transformed mouse fibroblasts is associated with the same nucleotide sequence. This result is the first direct evidence concerning the number of different transforming genes which exist in chemically transformed cells. Their number, at least in transformed mouse fibroblasts, may be very small.

A variety of different tumor cell lines have been screened with the intent of finding other types of transformed cells whose DNAs are able to induce transformation when applied to NIH 3T3 mouse fibroblasts. After extensive

screening, the DNAs from the following cell lines readily induced transformation: 1) a series of ethyl-nitrosourea-induced rat neuroblastomas; 2) a similarly induced glioblastoma; 3) a mouse Lewis lung carcinoma; and 4) human and mouse bladder carcinomas. It appears these DNAs can act across species and tissue barriers and that a variety of etiologic agents can be used to induce tumors having biologically active DNAs.

Studies are underway to clone the gene from a 3-MC transformed mouse fibroblast which encodes the transforming function. A cell in which the gene is closely linked to a fragment of the bacterial plasmid pBR322 has been created. Libraries of the DNA of this cell in the lambda phage vectors Charon 4a and 30 have been made. This library is currently being screened for components containing a pBR322 sequence. Isolation of such clones will allow not only cloning of the pBR322 sequence but also of the adjacently linked transforming gene.

Preliminary studies have been initiated to identify specific proteins of chemically transformed cells. Researchers have taken the DNAs of several bladder carcinomas and neuroblastomas, induced foci by transfection with these DNAs, cloned these foci and used the cells of these clones to induce tumors in newborn mice. The resulting tumors were in many cases rejected by virtue of an immune response of the host animal. However, the sera from these animals has been used for immunoprecipitation and the proteins of the resulting precipitates analyzed by gel electorphoresis. Preliminary, but well controlled, experiments indicate that these sera detect either bladder carcinoma specific or neuroblastoma specific proteins. More importantly, in both cases, the respective protein is found in the original donor tumor line as well as in the transfected derivative NIH 3T3 focus. In these two cases a protein may have been detected which is induced by a gene linked to the transforming gene. It is entirely possible that these proteins are encoded by the transforming genes themselves.

Mouse mammary tumor virus (MuMTV) is a type B retrovirus with at least three genes (gag, pol and env) on genomic RNA subunits of eight to nine kilobases. Although the viruses' generally poor activity in tissue culture has impeded biochemical study, MuMTV seems to replicate like other retroviruses and has the experimental advantages of causing mammary carcinomas in mice and showing dramatic regulation of viral RNA synthesis by glucocorticoid hormones in many types of infected cells. The mechanisms of carcinogenesis and steroidal regulation by this agent are poorly understood and analysis of integration sites of MuMTV DNA seemed likely to clarify these aspects and also the general mechanism of integration of retroviral DNA. Majors and Varmus, 1981, have determined the nucleotide sequence at host proviral junction of MuMTV by using the C3H strain of virus. Proviruses cloned from rat cells infected with MuMTV, a type B retrovirus regulated by glucocorticoid hormones, show the structural features of transposable elements; short inverted repeats conclude long direct repeats at the ends of viral DNA, and short sequences of cellular DNA are duplicated during integration and flank each provirus. The integrative mechanism joins a precise site in viral DNA to non-homologous sites in host DNA.

In the RNA virus studies II component, there are over 90 individual research projects being conducted on RNA tumor viruses. Of these, approximately 90% are predominately involved with studies with the avian tumor virus model systems. The remaining 10% touch on a variety of subjects such as phage work, plant viruses and other agents which may have some relationship, albeit more distant, to problems relating to human disease.

An important result of studies utilizing avian retroviruses as model systems takes advantage of their relatively simple genetic structure. It has been established that one of the four genes in the Rous sarcoma virus (RSV) carries all of the information necessary for the initiation of the transformed phenotype in cells infected with this virus. Studies of the gene product of this particular gene, called the $p60^{src}$ (or $pp60^{src}$), have demonstrated that this protein is a kinase. In addition, there are indications that other oncogenic retroviruses direct the synthesis of protein kinases. Several viruses which have this property include two feline sarcoma viruses, Fujinami sarcoma virus, which like RSV infects chickens, and the Ableson mouse leukemia virus.

It has been found that cells transformed by the Rous sarcoma virus contain levels of phosphotyrosine in proteins which are up to 50 fold greater than the levels present in uninfected cells. The importance of this kinase activity is also demonstrated by observations that in temperature sensitive transformation mutants of the Rous sarcoma virus, when the temperature is shifted to the permissive level a great increase appears in phosphotyrosine within one hour and a corresponding decrease occurs within an identical time-period when the temperature is shifted to the restrictive level. The obvious interpretation of these results is that modification of one or more cellular polypeptides catalyzed by $p60^{src}$ is crucial for cellular transformation by Rous sarcoma virus.

Phosphorylation of tyrosine is biologically a rare event, occurring in less than 0.5% of all phosphorylated amino acid residues. It was established that this phosphorylation occurs in vivo as well as in vitro in the Rous sarcoma virus system. RSV transformed cells contain cellular proteins which are newly phosphorylated on tyrosine residues. Several lines of genetic evidence indicate that the protein which modified these cellular proteins is $p60^{src}$. At least eight cellular substrates for $p60^{src}$ appear to exist in the RSV system. One of these, vinculin, is a previously identified polypeptide present in the specialized areas of the ventral surface of a normal cell which attaches it to the substratum. Vinculin is interesting because it is located in membrane structures called adhesion plaques which seem to play a role in the adherence of cells to surfaces. In addition the plaques serve as points of attachment between cells and anchor the actin filaments of the cytoskeleton to the inner side of the cell membrane. It has been suggested that the vinculin might link actin filaments to the membrane. It is known that disruption of actin filaments is one of the consequences of transformation and this is thought to contribute to the altered shape of the transformed cells. Moreover, transformed cells are usually much less adhesive than normal ones. Because of the finding that vinculin is a substrate for the kinase activity of the $p60^{src}$, it has been suggested that the phosphorylation of vinculin could destablize the linkage of actin filaments in the adhesion plaques, thus resulting in a disbursal of the filaments and weakening of the attachment of cells to surfaces. It has been recently

discovered that "src" gene kinase is present in adhesion plagues where it would have ready access to vinculin. It is attrative to speculate that modification of vinculin may play a role in the alteration of cellular morphology which occurs during transformation by RSV. It should be noted that there is no evidence of an increase in phosphotyrosine residues in proteins of mouse cells transformed by Moloney sarcoma virus, in Kirsten sarcoma virus transformed rat cells, in hamster cells transformed by Polyoma virus or in chick embryo cells infected with avian myelocytomatosis virus (MC 29), or in chemically transformed cells. This demonstrates that the increased phosphorylation of tyrosine does not necessarily result from all transforming events. Further, it suggests that all transforming proteins do not have activities identical to that of $p60^{src}$. Although the evidence is incomplete at this time, it seems unlikely that there is one universal biochemical basis for transformation.

Still another interesting finding resulting from studies on avian retroviruses concerns the relationship of polypeptide products of the transforming gene of RSV and the homologous gene of vertebrates. All vertebrate cells have been shown to contain a gene called "sarc" that has some homology with the transforming gene of Rous sarcoma virus (RSV). When the polypeptide products of the "sarc" gene ($p60^{sarc}$) of human, mouse and chicken cells are compared with the polymorphic polypeptide product of the "src" gene ($p60^{src}$) of several strains of RSV by two dimensional peptide mapping, the ($p60^{sarc}$) from chicken cells is clearly related to every viral ($p60^{src}$). Of the 13 methionine containing tryptic peptides of the $p60^{sarc}$ from chicken cells, 11 were present in some viral $p60^{src}$. Conversely, the other two peptides were not present in any $p60^{src}$. The eleven peptides from $p60^{sarc}$ of chickens that were shared with viral $p60^{src}$ were not all present, however, in any single viral $p60^{src}$. The 11 peptides from the $p60^{sarc}$ of chickens most closely resemble those in the $p60^{srcs}$ of B77 virus and the Prague stain of Rous sarcoma virus. Observations of this type are consistent with the hypothesis that cellular "sarc" is the progenitor of viral "src." In fact the $p60^{sarcs}$ of human, mouse and chicken cells are so similar in tryptic peptide composition that they are more closely related to each other than were some viral $p60^{srcs}$. The similarity of these maps suggests that the sequence of the $p60^{sarc}$ polypeptides has diverged very little during evolution. This may imply that the $p60^{sarc}$ is an essential cellular component.

A fundamental question is whether a cell infected with RSV is transformed because it contains too much of a normal cellular protein or because it contains an altered form of a normal cellular protein. The $p60^{sarc}$ and any of the viral $p60^{srcs}$ are similar in both structure and sequence. They are not identical, however. It is unknown at present whether the minor differences in structure observed are evidence merely of polymorphism of functionally similar proteins or whether they indicate that the cellular and viral proteins have different specificities. The answers to the question of functional equivalence can come only from the knowledge of the normal substrates for these two polypeptides in vivo.

Information of some significance to studies utilizing avian model systems are the recently published nucleotide sequence of an avian sarcoma virus oncogene (src) and the proposed amino acid sequence for the gene product. (Czernilofsky et. al., 1980). These investigators isolated the transforming

gene of avian sarcoma virus and adjacent regions of the viral genome, utilizing techniques of molecular cloning of the viral DNA. The nucleotide sequence of these isolated genes encompasses the whole "src" gene and a portion of the env gene that encodes gp70. It has been determined that the "src" gene encodes a single hydrophobic protein with structural features that confirmed previous descriptions of the gene product $pp60^{src}$. The molecular weight is 58,449. The protein is hydrophobic. Nine of the 12 methionines are in the carboxy half of the molecule and the amino acid sequence can account for seven sites of specific cleavage by protases predicted by previous work.

This protein consists of two separate functional domains: one which is confined to a molecular weight of about 15,000 at the amino terminus and anchors the protein in the cytoplasmic aspect of the plasma membrane, and the other domain protrudes from the membrane and carries the protein kinase activity. It is noteworthy that the functional domains have apparent correlates in the composition of the $pp60^{src}$ and that most of the protein is basic whereas the carboxy terminal domain is relatively acidic. It now seems apparent that the protein was designed on the one hand for tethering to the plasma membrane and on the other for enzymatic activity beyond the confines of this membrane. The portion of $pp60^{src}$ that is anchored to the plasma membrane contains one or more residues of phosphoserine whereas the exposed kinase domain contains phosphotyrosine.

Genetic loci responsible for neoplastic transformation of infected cells have been identified within the genomes of a number of nonavian viral systems such as Moloney murine sarcoma virus, polyoma virus, SV40 virus, and several strains of adenoviruses. A comparison of the nucleotide sequences reveals that "src" bears little relationship to the sequences of the other viral oncogenes with the exception of a few oligonucleotides shared with the gene for the middle T antigen of polyoma virus. There has been no appreciable homology found among the various amino acid sequences, although $pp60^{src}$, the T antigen of polyoma, and two proteins in the E1B region of adenovirus DNA are all relatively rich in proline.

The "src" gene is frequently deleted during the cultivation of most strains of avian sarcoma viruses and the mechanism until now has not been elucidated. It is currenly thought that the large repeating nucleotide sequence, termed D.R., that flanks the "src" gene might mediate deletion of a gene by homologous recombination within the viral genome. This is a hypothesis and has not as yet received experiemental verification.

The importance of this work is that the nucleotide sequence substantiates previous assumptions that "src" encodes a single protein. It can now be rigorously concluded that "src" is the only viral gene required for tumorogenesis by avian sarcoma viruses and that the mechanism of neoplastic transformation by the virus derives entirely from the biochemical properties of $p60^{src}$. To summarize the results of these studies, avian sarcoma viruses transform cells by virtue of synthesising proteins which are coded for by sequences which were originally derived from host cellular sequences. To date, three distinct sequences have been identified that have been acquired by these viruses: the "src" gene sequence, the fps sequence and the Y73 sequence. In two of these cases, and perhaps in all three, the sequences

code for protein kinases which phosphorylate tyrosine. Therefore studies on ASV seem to have revealed a central mechanism by which cells can be transformed.

The central features of transformation by avian viruses are shared with some mammalian viruses. Mammalian viruses that transform cells rapidly also appear to do so by virtue of acquired cellular sequences. All rapidly transforming mammalian retroviruses analyzed to date are replication defective and some of these acquired cellular sequences are expressed as "gag" related proteins. Examples of these would be the p120 of the Abelson murine leukemia virus which is also a plasma membrane located protein kinase which phosphorolates tyrosine, and the gag related proteins of the three feline sarcoma viruses, the p100 of the Gardner-Arnstein strain, the p78 of the Snyder Theilen strain and the p180 of the McDonough strain. There are some mammalian retroviruses in which the putative transforming protein is synthesized purely from acquired cellular sequences which contain no viral structural information, for example, the Harvey and Kirstin murine sarcoma virus p21 phosphoproteins. Similarly the mammalian retroviruses which cause leukemia only after a long clinical latency do so by mechanisms that have eluded investigations to date. Therefore, the general strategies of transformation by retroviruses seem to be similar regardless of the species of the virus.

Work in the area of hybridoma secreted monoclonal antibodies is ongoing in a number of laboratories. The work which may have some relevance to diagnostic procedures for human malignancies has been recently described by the Wistar group (Herlyn, et. al., 1981). Using mixed heamadsorption assays in quantitative adsorptions on a variety of malignant and nonmalignant cells, three of six hybridoma secreted antibodies bound to the majority of melanoma cell lines, melanoma tumors, and astrocytoma cell lines as well as to all normal and Epstein-Barr virus transformed lymphocytes. The binding patterns of these hybridomas coincide with the presence or absence of the D.R. antigen on human cells. However, two other antibodies, designated 19-19 and NU4B, detected two different antigens common to melanoma and astrocytoma cells only. Cloning of melanoma cells resulted in establishment of D.R. positive and negative clones with the binding of NU4B antibody retained in all cases. Thus, three hybridoma antibodies are specific for melanoma. Antibodies 19-19 and NU4B are quite specific for antigenic determinants common to most melanomas and some astrocytomas but to no other tissues studied. It is worth noting that, at least in the case of one patient, detection by monoclonal antibodies of melanoma antigens on the surface of tumor cells, freshly isolated or cultured in vitro, led to the reevaluation of histopathological diagnosis.

Workshops, conferences and courses supported by grants in FY 81 included the following: (1) Organization of the Cytoplasm, May 27-June 3, 1981, under the auspices of Cold Spring Harbor Symposium on Quantitative Biology (R13 CA 02809); (2) Cold Spring Harbor Cancer Research Courses (R13 CA 16224); in FY 81 these included: Animal Cell Culture in Serum Free Medium, June 13-26, 1981; Molecular Cloning of Eukaryotic and Viral Genes, June 30-July 20, 1981; Yeast Genetics, July 22-August 10, 1981; Molecular Biology of Plants, June 6-26, 1981; Introduction of Macromolecules into Mammalian Cells, July 22-August 10, 1981; and (3) the

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SUMMARY REPORT

DNA VIRUS STUDIES

DNA Virus Studies, supported by the contract mechanism concern research to explore the possible etiological relationship of DNA viruses to human malignancy and applied research to develop methods of diagnosis, prognosis, and intervention for neoplastic diseases associated with DNA viruses. The major effort during FY81 has centered on the Epstein-Barr virus (EBV) and the diseases with which it is associated.

Epstein-Barr virus directed nuclear antigen (EBNA) has been prepared from Raji cells to a purity of 90-95 per cent. Peptide mapping and cleavage studies have shown that the antigenically active 48K component, EBNA, has no peptides in common with the cellular proteins that co-purify with it in the early phases of purification nor with degradation products of the transformation-related 53K protein. The murine 53K protein present in SV40-transformed and in chemically induced mouse sarcoma cells is closely related to the human 53K component prepared from Raji cells. The host coded 53K protein is associated with the 48K DNA binding protein and is structurally similar since monoclonal antibodies to the 53K protein also bind to the 48K protein. Since discrimination between the two components is much better in human sera, human x human hybridomas are being established in order to aid in defining the association between these antigens and their roles in the transformation process.

In nature, EBV infects only B-lymphocytes and their derivatives, demonstrating that the EBV cell receptor is highly significant in controlling the host range of the virus. Nasopharyngeal carcinoma (NPC), however,is a malignancy of epithelial tissue and is associated with EBV, i.e., the tumor cells contain EBNA, the EBV genome in multiple copies, and the host presents a pattern of serological responses to EBV consistent with progression of the disease. The question asked then is: how does an epithelial cell lacking EBV receptors become infected with the virus? It is interesting to note that in NPC the probable primary site of the tumor is Waldeyer's ring, a structure in which the lymphoid tissue of the palatine and pharyngeal tonsils is contiguous with the epithelial tissue of the nasopharynx. Thus the processes currently under study include EBV DNA transfection; the use of hybrid virus, EBV particles in Sendai virus (SV) envelopes; and implantation of EBV receptors into the membranes of receptor negative cells. To date, attempts to transfect normal epithelial cells have not been successful. However, two epithelial cell lines both negative for EBNA and EBV DNA, one derived from a neck cancer and the other from a Chinese NPC patient, were transfected with total DNA from two EBV producer cell lines, HR-1 and AG-876, but not with viral DNA alone. Transfection was monitored by detection of early antigen (EA) and EBV DNA in the transfected cells. The hybrid virus (SV+EBV) was shown to bind to human T-lymphocytes and mouse spleen cells (neither of these possess receptors for EBV) and to "inject" EBV DNA into the cells. That EBV DNA was introduced into the cells was evidenced by a marked increase in the rate of DNA synthesis, but only when transforming virus, B95-8, was used. EBNA, for reasons not clear at present, could not be detected. Through the

use of reconstituted hybrid SV-Raji membrane vesicles, EBV receptors were implanted into the membranes of the recipient cells. Depending on the target cell, both transforming and nontransforming EBV could induce EBNA, early antigen (EA), and viral capsid antigen (VCA) to varying degrees. These successes will now permit studies not only on the biological activity of EBV in receptor negative cells, but also the effects of modified immunological properties of tumor cells.

Lymphoblastoid cell lines (LCL) in contrast to cell lines derived from Burkitt's lymphoma (BL) do not contain gp69/71, do not grow in soft agar, and are not tumorigenic in adult nude mice. Repeated cloning in soft agar resulted in a 2-6 fold increase in cloning efficiency and an increase in tumorigenicity. However, within 3 to 6 months both clonability in soft agarose and tumorigenicity declined to the background level of the parental lines. These studies affirmed agarose clonability as a relevant correlate of tumorigenicity. It was further shown that LCL can be induced by EBV superinfection to produce gp69. Whether such cells have also acquired a tumorigenicity marker has yet to be determined.

Previous studies have shown that BL derived cell lines containing multiple EBV genome copies vary in their response to interferon (IF); some lines are very sensitive to the growth inhibitory effect of IF whereas others are highly resistant. These variations may be due to: differences in normal B-lymphocytes, some occurrence during the process of neoplastic transformation, or an effect of in vitro establishment and propagation of the cell lines. In more recent studies of fresh biopsy tissue from nine BL patients, three were shown to be resistant to IF and six were sensitive in varying degrees. It is known that IF enhances natural killing (cytotoxicity) and also functions to induce resistance of target cells to natural killing; the latter effect occurring only in IF sensitive cells. It is possible then, that IF may protect the lymphoma cells against host rejection. The data suggest the importance of a better understanding of the complex interactions between IF, target cell sensitivity, and natural killing in the BL system.

In a study of very late relapses in BL patients, it was observed that 10 per cent of the patients may relapse after remission periods of one to six years (the duration of study). These patients maintained elevated early antigen-restricted type (EA-R) titers throughout their remission period, indicating that they were not out of danger as was confirmed by the later relapses. Whether the relapses are due to surviving BL cells or new tumor induction is not known. In the absence of detectable tumors, the elevated EA-R titers may be attributed to a highly active persistent EBV infection.

In a multicenter study of EBV serological markers for diagnosis and prognosis in patients with NPC, preliminary information indicates that antibody titers to EBV-induced membrane antigen (MA) measured by the antibody-dependent cellular cytotoxicity (ADCC) assay has probable prognostic value. The data show that high ADCC titers at diagnosis were indicators of a good prognosis following therapy, whereas low titers were indicators of poor

prognosis. The results also suggested that antibody to MA may function actively in vivo against these tumors. Since earlier studies had shown an inverse relationship between IgA antibody titers to VCA and ADCC titers, it was thought that the prognostic significance of ADCC titers in NPC patients may be related to the presence of IgA antibodies in the sera of these patients. Studies were undertaken to determine the role of IgA antibodies directed against EBV antigens in ADCC. It was found that IgA antibodies could not mediate ADCC against EBV-infected target cells; all ADCC activity resided in the IgG fraction. However, IgA antibodies were able to block the ADCC reaction mediated by IgG antibodies. This was shown by both pretreatment and direct competition experiments, and indicate that low ADCC titers in patients with NPC could be caused by the blocking of IgG-mediated ADCC by IgA antibodies. This suggests that high levels of IgA antibodies in the sera of NPC patients may be detrimental to the patient because of the blocking activity, if the assumption is made that ADCC functions in vivo against this tumor. It will be important now to determine whether the blocking activity can be overcome with sera containing high titers of ADCC activity. If this is the case, then serotherapy may be of some benefit to NPC patients whose sera contain high levels of IgA antibodies and low ADCC titers.

DNA VIRUS STUDIES

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CONTRACT NARRATIVES

DNA VIRUS STUDIES

Dr. Maurice L. Guss

ARMED FORCES INSTITUTE of PATHOLOGY (YO1-CP9-0500)

Title: Application of Epstein-Barr Virus Markers to Diagnosis and Prognosis of Nasopharyngeal Carcinoma and Occult Tumors of the Nasopharynx Area in U.S.A.

Contractor's Project Director: Dr. Vincent Hyams

Contractor's Project Officer (NCI): Dr. Robert Goldberg

Objectives: The contractor provides support to the Mayo Foundation, NO1-CP9-1006, by supplying clinical information and materials and histopathology consultation.

Major Findings: The contractor has enrolled 26 nasopharyngeal carcinoma patients in the collaborative study.

Significance to Biomedical Research and the Program of the Institute: This project provides support for a contract to demonstrate that Epstein-Barr virus assays can be used for improved diagnosis and management of American patients with head and neck tumors. It is anticipated that the knowledge gained could be transferred to cancer centers for wider application in cancer control. The study will further permit assessment of the specificity of the viral markers in the identification of high risk populations and assessment of other environmental factors contributing to the etiology of nasopharyngeal carcinoma.

Proposed Course: This project will continue without change.

Date Contract Initiated: November 28, 1978

Current Annual Level: $1,500

CALIFORNIA, UNIVERSITY OF, LOS ANGELES (NO1-CP0-1022)

Title: Studies of Interrelationship of Viruses, Genetics and Immunity in the Etiology of Human Cancer.

Contractor's Project Director: Dr. Mitsuo Takasugi

Project Officer (NCI): Dr. Paul H. Levine

Objectives: (1) To detect cellular and humoral immunity in cancer patients and to determine the specificity of these reactions; (2) to understand the cellular components of the immune response to cancer and its interaction with antibodies in terms of resistance to susceptibility to cancer; and (3) to study the immune response of cancer patients and normals to different viruses and virus-induced antigens.

Major Findings: Sera from 40 patients with Burkitt's lymphoma and 64 patients with nasopharyngeal carcinoma were tested for specific antibody dependent cell cytotoxicity (ADCC) activity against Epstein-Barr virus (EBV)-associated antigens on EBV-infected target cells. Average activity was calculated for sera from each patient and the results were analyzed for correlations with survival. Patients were divided by ADCC titers into two groups: those with titers greater than 2.50 and those with titers less than 2.50. A significant difference in survival for the two groups was observed. When the same patients were divided into quartiles by ADCC activity, only the group with the weakest activity survived for a significantly shorter time than the others.

Survival was also analyzed by age, sex, and stage. When patients were divided into two age groups of 4-10 and 11-20, a difference in survival was observed between younger and older patients with the younger patients surviving longer. However, the older patients may have been mostly in later stages of the disease when sera were obtained. No significant sex-associated differences in ADCC activity were observed although the survival curve for males was slightly higher than that for females. More patients of both sexes need to be studied to clarify this question. When patients were investigated by the stage of disease, only patients in stage four survived for a significantly shorter period. ADCC for this group was also significantly lower.

Significance to Biomedical Research and the Program of the Institute: Studies in progress may help to determine whether certain groups of people have specific responses to EBV or other suspected tumor viruses. The studies are likely to provide useful techniques for studying antigenic expression and modulation following virus infection or transformation, and in the diagnosis, prognosis and treatment of various forms of cancer.

Proposed Course: This contract terminated on July 31, 1981.

Date Contract Initiated: July 12, 1971

Current Annual Level: $139,570 (7 months)

CALIFORNIA, UNIVERSITY OF, LOS ANGELES (NO1-CPO-1024)

Title: Studies of HLA Genetic Markers of Immune Response to Cancer Viruses.

Contractor's Project Director: Dr. Paul I. Terasaki

Project Officer (NCI): Dr. Paul H. Levine

Objectives: To examine the relationship of genetic markers (HLA and others) for linkage to the incidence of cancer in families, and to characterize B lymphocyte alloantigens and to determine their expression in cell hybrids and changes in cells following virus infection or transformation.

Major Findings: A number of cancers were examined for possible immunue associated phenotypic markers, particularly HLA-DR antigens. One study compared 66 breast cancer patients with 218 normal controls. There was a statistically significant decrease in DR2 in the patient group. In another study of breast cancer patients, HLA testing was done to check for possible genetic markers for rapidly progressing breast cancer. No differences were found between patients and controls. Other cancers examined include malignant melanoma (no differences between 236 patients and 575 controls), colon carcinoma, and sarcoma of soft tissue. In a study of 99 Japanese stomach cancer patients against 222 controls, no significant differences in antigen frequencies were found. In addition to cancer patient studies, the nature of the antibodies involved has been investigated. Reactivity of cold-reacting non-HLA cytotoxins in particular seems to be related to the amount of surface immunoglobulin on the target cells. Inhibition and stripping tests indicate that the target antigen is IgM in nature. Monitoring of different cell types was greatly enhanced by development of refined electronic sizing of leukocyte subpopulations.

Significance to Biomedical Research and the Program of the Institute: The identification of disease susceptibility genes is of great importance to studies on the etiology of cancer. In family studies, linking genetic type and immunologic response to tumor-and virus-associated antigens may help to determine which humans are more likely to develop specific tumors as well as which abnormal immune responses to certain viral antigens are closely linked to tumors.

Proposed Course: This contract terminated on December 31, 1980.

Date Contract Initiated: July 12, 1971

Current Annual Level: No Funds in FY81

CENTRE NATIONAL de la RECHERCHE SCIENTIFIQUE (NO1-CP9-1035)

Title: Comparison and Evaluation of IgA Antibody Levels to Epstein-Barr Virus-VCA in Nasopharyngeal Carcinoma Patients from High, Intermediate, and Low Risk Populations.

Contractor's Project Director: Dr. Guy de Thé

Project Officer (NCI): Dr. Maurice L. Guss

Objectives: The objective is the determination of the usefulness of IgA antibody to Epstein-Barr virus (EBV) in the diagnosis and prognosis of nasopharyngeal carcinoma (NPC) in low, intermediate, and high risk populations. The study of three regions of differing disease frequency will provide information on the relationship of environmental factors to disease and on the significance of low VCA titers in healthy family members.

Major Findings: During this study period the clinically oriented NPC program progressed satisfactorily. In Paris, the NPC patient accession was 100, 15 being European. In Hong Kong, the recruitment reached 73 patients. The follow-up of patients is being satisfactorily conducted in Villejuif and Hong Kong and great efforts are being made in Tunis to select only the patients for whom follow-up will be feasible.

A new probe was developed using internal repetitive sequences of the EBV DNA to search for EBV DNA genomes. This probe was used to test the biopsies collected and permitted detection of the presence of EBV DNA in two of four well differentiated carcinomas of the nasopharynx. Special efforts to collect more well differentiated carcinomas from different geographical areas were undertaken in order to assess the proportion of EBV-positive to EBV-negative cases.

Of 13 nonNPC tumors of the nasopharynx (including eight lymphomas collected from adult Parisians), none showed an association with EBV based on both serology and EBV DNA markers.

Among 420 blood-linked relatives of 122 NPC patients in Hong Kong, IgA/VCA antibodies were detected in ten individuals. Among these ten cases, six permitted tissue biopsy, and of these, three were found to have NPC at stage I.

Significance to Biomedical Research and the Program of the Institute: This project offers the opportunity to demonstrate that EBV assays can be used for improved diagnosis and management of patients with head and neck tumors. It is anticipated that the knowledge gained could be transferred to cancer centers for wider application in cancer control. The study will further permit assessment of the specificity of the viral markers in the identification of high risk populations and other environmental factors contributing to the etiology of NPC.

Proposed Course: This project will continue without change.

Date Contract Initiated: August 16, 1979

Current Annual Level: $187,000

CHILDREN'S HOSPITAL OF PHILADELPHIA (NO1-CP3-3272)

Title: The Propagation and Seroepidemiology of Epstein-Barr Virus.

Contractor's Project Director: Dr. Gertrude Henle

Project Officer (NCI): Dr. Maurice L. Guss

Objectives: To investigate the relationship between Epstein-Barr virus (EBV) and human cancer. Studies on this contract include: (1) improvement of existing, and development of new, techniques for the detection of EBV-related antigens and titration of corresponding antibodies; (2)

search for methods to measure cell-mediated immune reactions in EBV-associated diseases, and, if successful, their application to detection of blocking (tumor enhancing) factors; (3) determination of frequencies and titers of antibodies to various EBV-determined antigens in EBV-associated diseases providing prognostic information and support for a causal relation of EBV to given human malignancies.

Major Findings: Study of very late relapses in Ghanaian Burkitt's lymphoma (BL) cases after remission periods of over 12 and as many as 78 months showed that the patients maintained high titers of antibodies to the R component of the EA complex, confirming the prognostic implications of anti-R. Retrospective studies of Ugandan BL patients revealed that initial tumor burden and anti-EA tests provide the most dependable prognostic information. Antibodies neutralizing EBV-specific DNase were found to be another outstanding feature of nasopharyngeal carcinoma (NPC). Sera collected from six Alaskan natives more than three years before diagnosis of NPC yielded no clues as to their future fate; another patient showed the serologic pattern characteristic of NPC 21 months before diagnosis when he probably already had the tumor. Thus, the EBV-specific serology can identify NPC patients before they seek medical aid. Collaborative studies on NPC in American and European children and adults have shown without exception an association of EBV with this undifferentiated or nonkeratinizing carcinoma and the correlation of antibody patterns with the histology. Chronic inflammatory or metaplastic lesions present in the nasopharynx of NPC patients may be EBV-associated as may also be salivary tumors although it has not been determined whether the viral genomes were in normal or tumorous salivary cells or in the lymphoid elements present in the tissue.

Studies on cellular immune responses in infectious mononucleosis (IM) are progressing. While primary EBV infections in early childhood remain mostly silent, typical cases of IM can be identified occasionally in infants, the youngest was 10 months of age. At an early age the incidence of heterophil antibody responses is reduced and their titers increase with age. Three of 18 children with Reye's syndrome gave serologic evidence of current or very recent primary EBV infections.

Studies on humoral and cellular immune responses in immunologically compromised patients have been continuing in order to identify the defect(s) responsible for the observed elevated anti-VCA and anti-EA titers and low or nondetectable anti-EBNA levels. These efforts also include patients with Hodgkin's disease and other lymphomas, ataxia telangiectasia, and renal or marrow transplants. It seems that the EBV-specific serology might provide another parameter for assessment of cellular immunity. Most intriguing, B cell lymphomas arising as enhanced frequency in these types of patients were associated with EBV.

Significance to Biomedical Research and the Program of the Institute: The primary purpose of these studies is to aid in the determination of the etiologic relationships of EBV to certain human malignancies. Fingerprints of EBV have been found in nearly all BL and NPC biopsies. EBV-related serology may serve to detect advancing disease, to provide prognostic information, and to monitor the effectiveness of therapy.

Proposed Course: Continue improvement of existing assays (particularly EBNA) and the development of new assays for the measurements of humoral and cell-mediated immunity to EBV-associated antigens; conduct continued longitudinal studies on African BL patients to provide further evidence for the prognostic significance of EBV-related antibody patterns and to determine, especially, the relationship of anti-EBNA titers to clinical events; determine the relationship of EBV titers and T-cell function in studies on renal transplantations and IM.

Date Contract Initiated: March 1, 1973. This is a continuation of the Contract PH 43-66-477 initiated February 2, 1966.

Current Annual Level: $186,616

GLASGOW, UNIVERSITY OF (NO1-CP8-1022)

Title: Environmental Carcinogens and Papilloma Viruses in Cattle Cancer.

Contractor's Project Director: Professor W. F. H. Jarrett

Project Officer (NCI): Dr. Maurice L. Guss

Objectives: Four types of bovine papilloma viruses will be characterized. Papilloma virus genomes will be sought in alimentary squamous cell carcinomas, adenomas, and adenocarcinomas in the intestines of cattle from bracken fern-infested farms. Studies will be conducted to gain insight into the nature of the mechanism of interplay of bracken fern components and papilloma viruses.

Major Findings: Studies have been continued on the four bovine papillomaviruses (BPV) which have already been characterized by the contractor. BPV-1 is the teat frond and penis virus which may spread to skin. BPV-2 is the classical cutaneous fibropapillomavirus; BPV-4 is the alimentary virus; and BPV-5 is the teat rice grain virus. A large number of papillomas has been studied and the viruses appear to have the broad lesion site and histopathological specificity indicated earlier. Further cross-hybridization and immunological studies indicate that there is a much closer relatedness between BPV-1 and BPV-2 than between the other two. BPV-2 has been cloned in *E. coli* and probes prepared. While corresponding sequences have been demonstrated in skin papillomas and a variety of transformed fibroblasts, no sequences of this virus have been detected in carcinomas. The other viruses are now being cloned. Fully differentiated epithelial tissue cultures have been infected with BPV-4 and attempts are being made to increase virus output and to study the effect of added plant compounds on possible transformation. In the animal experiments, a second season of bracken and azathioprine feeding has been carried out. Marked hematological changes are present in several groups and flagrant massive pharyngeal papillomatosis is present in the azathioprine plus virus group. Possible evidence for latency of papillomavirus has been found.

Significance to Biomedical Research and the Program of the Institute: Epidemiological data indicate a possible association between wart viruses and the subsequent development of certain neoplastic tumors. This unique animal model system lends itself to observations on the natural interaction of virus and carcinogen resulting in malignant disease. This effort may provide leads to analogous human diseases such as familial polyposis and papillomatosis of the human bladder.

Proposed Course: This project will terminate on September 10, 1981.

Date Contract Initiated: September 11, 1978

Current Annual Level: No Funds in FY 81

GOTHENBURG, UNIVERSITY of (NO1-CP8-1020)

Title: Studies on the Biomolecular Relationship of Herpesviruses (Epstein-Barr Virus) and Cancer.

Contractor's Project Director: Dr. Tomas Lindahl

Project Officer (NCI): Dr. George Vande Woude

Objectives: To characterize the Epstein-Barr virus (EBV) genome and compare the latent EBV genomes present in a series of human cell lines derived from patients with various diseases associated with EBV; and to determine whether specific EBV strains are correlated with certain malignant diseases.

Major Findings: EBV DNA from B95-8 virions has been cleaved with restriction endonucleases BamHI and EcoRI, and the resulting fragments cloned in the plasmid pBR322. The largest BamHI fragment is $8X10^6$ MW, and about 25 different fragments of $1-8X10^6$ MW have been cloned. This represents the large majority of the sequences in the EBV genome. Moreover, all EcoRI fragments smaller than $MW=6.10^6$ have been cloned in pBR322. The largest EcoRI fragments are too long for cloning in this vector, but have instead been cloned in the cosmids Homer 1 and pJC79. Restriction enzyme digests of circular EBV DNA from Raji cells are used to obtain the end fragments of the viral genome in cloned form. ^{32}P-labeled or ^{125}I-labeled probes of the cloned EBV DNA sequences have been prepared by nick translation with E. coli DNA polymerase I. These probes are being used to characterize the sequence complexity of the EBV DNA present in transformed cell lines containing small amounts of viral DNA.

The restriction enzyme cleavage patterns of several EBV isolates have been analyzed by the Southern blotting technique. Circular, intracellular EBV DNA from the lines Raji, NC-37, and F-265 show identical cleavage patterns with the BamHI, HindIII, and EcoRI enzymes, strongly indicating that the latter two lines are not really derived from normal individuals but are sublines of Raji. The Southern blotting technique is also used to characterize the sequence complexity of a distinct, physically separable minority of EBV DNA sequences from Raji cells with the properties of linearly integrated DNA.

Significance to Biomedical Research and the Program of the Institute: Recent advances in biochemical techniques for segregating and characterizing small DNA fragments released by endonucleases provide the opportunity to assay tumor tissues for the presence of a small fraction of herpesvirus genome in the host cell genome. A major objective is the determination of etiology of diseases which so far have been shown to be associated with specific DNA viruses by serological and epidemiological techniques. The new capability to extend present knowledge to include biochemical genetic information about the relationship of specific DNA viruses to specific cancers has a high probability of contributing to knowledge of cancer etiology.

Proposed Course: This project terminated on October 31, 1980.

Date Contract Initiated: October 31, 1977

Current Annual Level: No Funds in FY81

HARVARD UNIVERSITY (NO1-CP8-1005)

Title: Biomolecular Relationship of Herpesviruses (*Herpesvirus saimiri*) and Cancer.

Contractor's Project Director: Dr. Carel Mulder

Project Officer (NCI): Dr. George Vande Woude

Objectives: The viral genome structures of four different strains of *Herpesvirus saimiri* (HVS) of varying oncogenic potential will be analyzed and compared using restriction endonucleases. HVS DNA and specific DNA fragments will be tested in vitro for transforming potential in isolated lymphatic primate cells and in vivo for their potential to induce tumors.

Major Findings: HVS DNA sequences in several transformed lymphoblastoid cell lines were analyzed. A small portion of the viral DNA in nonproducer cell lines occurs in circular episomal form; the remainder is in the linear cellular DNA. The episomal DNA appears to lack several *EcoRI* fragments; this may be due to an inability of detection of very small amounts or a sequence arrangement differing from the standard. Producer cell lines, on the other hand, contain all of the viral restriction fragments as seen in virion DNA, and additionally may contain fragments of unknown origin.

Studies have continued to elaborate the precise sites of methylation in left end of the unique HVS L-DNA of nonproducer cell lines. This may provide some insights on the role of methylation in the establishment of a latent, nonpermissive infection.

Significance to Biomedical Reseach and to the Program of the Institute: Recent advances in biochemical techniques for segregating and characterizing small DNA fragments released by endonucleases provide the opportun-

ity to assay tumor tissues for the presence of a small fraction of herpesvirus genome in the host cell genome. A major objective is the determination of etiology of diseases which, so far, have been shown to be associated with specific DNA viruses by serological and epidemiological techniques. The new capability to extend present knowledge to include biochemical genetic information about the relationship of specific DNA viruses to specific cancers has a high probability of contributing to cancer etiology.

Proposed Course: This project terminated on December 31, 1980.

Date Contract Initiated: January 1, 1978

Current Annual Level: No Funds in FY81

ILLINOIS, UNIVERSITY OF (NO1-CP7-1061)

Title: Integration Sites of Papovavirus Genomes in Transformed Cells.

Contractor's Project Director: Dr. Kiranur Subramanian

Project Officer (NCI): Dr. Maurice L. Guss

Objectives: Construction of physical maps of Simian Virus 40 (SV40) DNA integrated in the genome of transformed cells, and determination of the nucleotide sequences at the junction of cell DNA and the integrated SV40 DNA.

Major Findings: The research concerns a viable mutant of SV40, in1449. The mutant DNA is found to have an 157 nucleotide-long insertion at map position 0.649 within the 5' untranslated sequence of the early region of SV40. Sequence comparisons show that the insert is not of SV40 origin, but probably of monkey origin since in1449 was produced in monkey kidney cells.

The sequence of the in1449 insert matches remarkably well with sequences of a predominant family of interspersed repeated sequences in human DNA (called the Alu family) and their cloned members. The high degree of sequence homology suggests that the in1449 insert is derived from a member of a family of interspersed repeated sequences in monkey DNA related to the human Alu family. The in1449 insert and the Alu family contain oligonucleotide sequences that are also conserved in the replication origins of papovaviruses and in repetitive double-stranded regions of mammalian heterogeneous nuclear RNAs.

Sequences around the two recombinant joints in in1449 exhibit a definite pattern of homology. An octanucleotide present in the SV40 part of the first recombinant joint is exactly repeated 15 nucleotides away within the insert; another octanucleotide present within the insert at the second joint is exactly repeated 21 nucleotides away in the viral DNA. The viral DNA sequences flanking the insert in in1449 also exhibit some homology. These data suggest site-specificity in the two recombinational events leading to the production of the in1449 mutant.

The monkey origin of the in1449 insert and its presence as a repeated sequence within the monkey genome have been confirmed by hybridization studies. The hybridization efficiency of human and monkey Alu family-type sequences to the in1449 insert are about the same, showing again that they are practically identical. The evolutionary relationship of Alu family-type interspersed repeated sequences has been compared among various vertebrates. Rat, calf and chicken sequences exhibit weak homology with the human and monkey sequences, and the homology is very poor in the case of frog and salmon sperm sequences versus monkey sequences; invertebrate sequences from Drosophila exhibit practically no homology at all.

Significance to Biomedical Research and the Program of the Institute: Examination of the interactions between genomes of eukaryotic cells and oncogenic viruses may identify the basic reactions of cell regulation which control expression of viral-genetic information or which are deranged by the virus and result in transformation to malignancy.

Proposed Course: This project terminated on October 12, 1980.

Date Contract Initiated: September 29, 1977

Current Annual Level: No Funds in FY81

INDIAN HEALTH SERVICE (YO1-CP9-0501)

Title: Application of Epstein-Barr Virus Markers to Diagnosis and Prognosis of Nasopharyngeal Carcinoma and Occult tumors of the Nasopharynx Area in U.S.A.

Contractor's Project Director: Dr. Anne Lanier

Contractor's Project Officer (NCI): Dr. Robert Goldberg

Objectives: This contractor will provide support to the Mayo Foundation, NO1-CP9-1006, by supplying clinical information and materials.

Major Findings: The Indian Health Service has enrolled 22 nasopharyngeal carcinoma patients in the collaborative study.

Significance to Biomedical Research and the Program of the Institute: This project provides support for a contract to demonstrate that Epstein-Barr virus assays can be used for improved diagnosis and management of American patients with head and neck tumors. It is anticipated that the knowledge gained could be transferred to cancer centers for wider application in cancer control. The study will further permit assessment of the specificity of the viral markers in the identification of high risk populations and other environmental factors contributing to the etiology of nasopharyngeal carcinoma.

Proposed Course: This project will continue without change.

Date Contract Initiated: November 30, 1978

Current Annual Level: $26,920

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (NO1-CP9-1009; successor to NO1-CP4-3296)

Title: Sero-Epidemiologic and Laboratory Studies on Nasopharyngeal Carcinoma and Burkitt's Lymphoma.

Contractor's Project Director: Dr. C. A. Linsell

Project Officer (NCI): Dr. Maurice L. Guss

Objectives: To conduct epidemiological studies of virus associated cancers.

Major Findings: Field work in the West Nile District was terminated in January 1980. Sera from two pre-bled Burkitt's lymphoma (BL) cases which were detected after the 1976 testing were investigated for Epstein-Barr virus (EBV) antibody activities. The results showed that the EBV-VCA titers were significantly higher in the pre-bled sera, thus further supporting the hypothesis that it is early and heavy EBV infection that promotes BL development.

The continued BL case detection in 1979 showed that the incidence is low, both in North and South Mara. The malaria suppression in North Mara failed to bring the prevalence of parasitemia down to the target level. In order to find the reason for this failure, chloroquine sensitivity testing of local malaria parasites was carried out in Shirati. The results showed that malaria parasites in the study area continue to have the same level of chloroquine sensitivity as is usually found in East Africa. Investigations in the participating villages showed that approximately 75 per cent of the eligible children regularly received their chloroquine tablets, which indicates that the population's cooperation in the scheme is as active as can possibly be obtained in any community-wide medication program.

HLA typing of newly diagnosed cases of NPC and controls from the three major Chinese dialect groups in Singapore has continued. The NPC Register has been entered into the IARC computer to enable HLA genotypes in terms of A2, B17, and BW46 to be determined.

Significance to Biomedical Research and the Program of the Institute: EBV is a naturally-occurring virus strongly suspected of an etiologic role in human cancer. The research under this contract should help elucidate the role of EBV in the Burkitt's lymphoma. Studies of blood genetic types, together with the sero-epidemiological results, may provide the means for detecting NPC high risk groups among a normal population.

Proposed Course: In Africa, case detection will continue to have high priority in order to determine the relationship of EBV antibodies to subsequent appearance of disease. The NPC program will be continued to define the genetic factors relating to disease.

Date Contract Initiated: April 1, 1979

Current Annual Level: $81,970

KAROLINSKA INSTITUTE (NO1-CP3-3316)

Title: Studies on the Significance of Herpes-Type Viruses and RNA Viruses in the Etiology of Some Human Cancers

Contractor's Project Director: Dr. George Klein

Project Officer (NCI): Dr. Maurice L. Guss

Objectives: To elucidate Epstein-Barr virus (EBV)-cell-host interactions and mechanisms of cell-mediated antitumor immune reactions.

Major Findings: The Epstein-Barr virus determined nuclear antigen (EBNA) is uniquely and regularly expressed in all EBV transformed cells and in all Burkitt's lymphoma (BL) and nasopharyngeal carcinoma (NPC) tumors. Additionally EBNA is a DNA-binding protein, showing many analogies with the T-antigen of the papovaviruses. Closely associated with EBNA in EBV-cell lines is a cellular 53K DNA-binding protein. This component, also found in EBV-negative lymphoma lines parallels the 53K protein found associated with T-antigen in cells transformed by papovaviruses and adenoviruses, and in non-T-antigen associated chemically induced sarcomas and spontaneous teratocarcinomas. The contractor has shown that, by peptide mapping, the 48K protein of purified EBNA is not a degradation product of the 53K component; and that the 48K protein, but not the 53K, gives an EBNA positive staining reaction and inhibits the EBNA reaction with standard target cells. Analogous to SV40 transformed cells, EBV converted cells contain increased numbers of DNA replication forks, thus indicating a role for initiation of DNA synthesis by EBNA similar to SV40 T-antigen. Similarly, microinjection of quiescent 3T3 cells with EBNA led to pronounced stimulation of DNA synthesis.

The contractor demonstrated that EBV receptors could be transplanted to membranes of receptor negative cells, using Sendai virus envelopes as the vehicles. EBV-exposed receptor-implanted cells were shown to contain 50 to 75 per cent of the tritiated EBV DNA label 24 hours after infection. The viral genome was shown to be functionally active by expression of EBNA, EA, and VCA in B lymphocyte cell lines of human, murine, and baboon origin; in T lymphocyte cell lines of human and murine origin; in mouse fibroblasts; and in freshly explanted mouse lymphocytes. These studies not only indicate that the severe host range restriction of the virus may be primarily determined at the receptor level, but that research on mechanisms responsible for latency and tumorigenicity of the virus may now be done more easily in the laboratory through the use of cell lines from small rodents.

Significance to Biomedical Research and the Program of the Institute: Investigations under this project are directed to two areas of importance to overall Program. First, the recognition that certain herpesviruses induce neoplasms in animals and that EBV is associated with human neoplasms requires intensive study to provide a better understanding of the host-virus relationship for this group of agents. Data acquired under this project contribute to assessment of the role of herpesviruses in the causation of human neoplasms. Second, the analysis of the immunological

responses of the host to tumor cell surface antigens provides basic information important in approaches to control of tumor development. The project is strongly oriented to human neoplasia, using defined animal systems as required for progress in understanding the fundamental mechanisms involved.

Proposed Course: This project will terminate on July 31, 1981.

Date Contract Initiated: April 9, 1968

Current Annual Level: $72,000 (4 months)

MASSACHUSETTS GENERAL HOSPITAL (NO1-CP4-3222)

Title: Activation of Oncogenic Viruses and Induction of Cancer by Immunologic and Nonimmunologic Methods.

Contractor's Project Director: Dr. Martin S. Hirsch

Project Officer (NCI): Dr. Maurice L. Guss

Objectives: To determine the effects of human interferon in the prophylaxis of virus infections in immunosuppressed kidney transplant patients.

Major Findings: Double-blind, placebo-controlled clinical trials of human leukocyte interferon (IFN-α) in renal transplant recipients are being conducted. In the initial study, 3.0×10^6 units of IFN-α administered twice weekly for six weeks was shown to delay the onset of cytomegalovirus (CMV) and Epstein-Barr virus (EBV) excretion, as well as to reduce CMV viremia. Patients enrolled in this study continue to be followed for late sequellae of infection or treatment, particularly neoplasia. No tumors have yet developed and graft survival is equivalent in IFN-α and placebo groups. During the past six months, the effects of IFN-α on JC papovavirus infections in this population have been evaluated.

Two new studies have been undertaken in an effort to expand information concerning IFN-α viruses, and cancer in the high risk renal transplant population. One involves patients at risk for primary CMV infection (seronegative recipients of kidneys from seropositive donors), and is a multicenter collaborative trial. The second involves patients susceptible to reactivation CMV infection (seropositive recipients). In both trials, treatment courses have been extended to 14 weeks and total IFN-α doses have been increased from 36 million to 102 million units. The mechanisms of IFN-α induced protection in these patients are being studied by performing pharmacokinetic and immunological function studies on individual recipients.

Significance to Biomedical Research and the Program of the Institute: The studies on the inhibition of virus infection in immunosuppressed patients should provide valuable data on interferon prophylaxis, which may later be more directly applicable to cancer prophylaxis and therapy.

Proposed Course: The current clinical studies will be continued according to the protocols already established, with appropriate modifications as these seem clinically indicated. Plans have been developed in collaboration with other renal transplant centers to expand the studies of interferon, viruses and cancer in kidney graft recipients. Particularly high risk groups will be studied and regimens will include higher dose interferon for more prolonged periods.

Date Contract Initiated: September 15, 1971

Current Annual Funding: $207,000

MAYO FOUNDATION (NO1-CP9-1006)

Title: Application of Epstein-Barr Virus Markers to Diagnosis and Prognosis of Nasopharyngeal Carcinoma and Occult Tumors of the Nasopharynx Area in U.S.A.

Contractor's Project Director: Dr. Gary Pearson

Project Officer (NCI): Dr. Robert Goldberg

Objectives: The objective of the project is the determination of the usefulness of Epstein-Barr virus (EBV) markers in established serological tests for diagnosis and prognosis of nasopharyngeal carcinoma (NPC) and primary tumors of the head and neck in the United States.

Major Findings: Twenty-seven new patients with suspected or confirmed NPC were added to the study during this reporting period. This brings the total number of cases to 153 over the two-year period this contract has been in effect. Among the 153 patients, NPC has been confirmed histopathologically in 121 patients. Clinical data is complete on most of these patients except for long-term follow-up. In addition, sera from 76 patients with occult tumors have now been serologically tested. Sera from a large number of control groups including 150 patients with lung cancer were also examined during this reporting period. All sera were examined for antibodies to EBV antigens by immunofluorescence in two different laboratories (Henle, Pearson) and for antibodies capable of mediating antibody dependent cellular cytotoxicity (ADCC). The antibody titers were then related to stage of disease at diagnosis and histopathology. IgA antibodies to viral capsid antigens (VCA) and IgG antibodies to EBV-induced early antigens (EA) were present at a high frequency and at generally elevated titers in patients with nonkeratinizing (WHO II) and poorly differentiated NPC (WHO III) in comparison with well-differentiated squamous cell carcinomas (WHO I) and the various control groups. These findings still indicate, therefore, that these two tests are potentially useful diagnostic aids for these two histopathological types of this disease as determined by four different staging methods and antibody titers to EBV antigens. These procedures have also been useful for identifying NPC in eight patients that were initially occult but eventually confirmed by biopsy. All patients are being followed to determine the prognostic value of EBV serology. In those patients that have died of disease so far, increases in anti-EBV antibody titers generally were

detected before death. The ADCC findings so far appear to be the best predictor of disease course although it is still to early to draw conclusions on this point. In addition, a number of biopsies have been examined for EBV by the hybrization or EBNA procedures. Fifteen NPC biopsies out of 20 studied to date have been positive for EBV by at least one of these assays. All positive NPC biopsies with one exception were from WHO II or WHO III histopathological types. In addition, two biopsies from Alaskan patients with lymphoma in the nasopharynx were positive for EBV genomes by the hybridization procedure. HLA and DR typing have been completed on lymphocytes from 49 and 29 patients respectively. The data to date suggest that there might be meaningful differences between patients and controls at the B5, B27 and B40 loci.

Significance to Biomedical Research and the Program of the Institute: This project offers the opportunity to demonstrate that EBV assays can be used for improved diagnosis and management of American patients with head and neck tumors. It is anticipated that the knowledge gained could be transferred to cancer centers for wider application in cancer control. The study will further permit assessment of the specificity of the viral markers in the identification of high risk populations and other environmental factors contributing to the etiology of NPC.

Proposed Course: This project will continue without change.

Date Contract Initiated: October 23, 1978

Current Annual Level: $290,000

OHIO STATE UNIVERSITY (NO1-CP8-1021)

Title: Studies on the Epstein-Barr Virus and its Association with Nasopharyngeal Carcinoma.

Contractor's Project Director: Dr. Ronald Glaser

Project Officer (NCI): Dr. Maurice L. Guss

Objectives: Determination of the role of Epstein-Barr virus (EBV) in nasopharyngeal carcinoma (NPC) by conducting studies on (1) infection by EBV of normal human epithelial cells, particularly nasopharyngeal cells; (2) cellular hybridization with an HR-1 cell line containing one EBV genome per cell; (3) transformation of normal human primary epithelial cells with EBV by a nuclear exchange procedure; and (4) in vivo cell fusion of EBV genome positive lymphoid cells and nasopharyngeal cells.

Major Findings: Studies have been continued in attempts to transfect human epithelial cells with EBV DNA. A second human epithelial (carcinoma) cell line has been transfected with DNA derived both from HR-1 and AG876 lymphoblastoid cells. EBV DNA derived from the AG876 cell line, which produces transforming virus, is capable of inducing

lytic infection in the two different epithelial cell lines that were used. EBV DNA derived from these two cell lines behaved differently in the two transfected epithelial cell lines, i.e., the HR-1 derived virus DNA was restricted to only EA synthesis in one of the two epithelial cell lines, but similar DNA preparations derived from AG876 cells used to transfect the same cell lines were able to induce both EA and VCA. In studies regarding possible co-carcinogenic effects of EBV and malaria in owl monkeys and marmosets, it was observed that infecting common marmosets with malaria parasites seems to depress the cellular immune response in regard to natural killer cell activity, when compared to EBV infected and control monkeys. There is also some evidence to suggest that coinfection of these monkeys with both malaria and EBV may suppress the humoral response of the animals against EBV specific antigens. Data from collaborative studies with two hospitals in the People's Republic of China show that: (1) EBV specific IgA antibodies are a good marker for differentiating between normal and NPC patients; (2) antibody titers against EA and VCA are elevated in NPC patients when compared to controls; (3) antibody against the EBV DNase enzyme still continues to be an excellent marker for NPC; and (4) a new assay in which abnormal nucleosides in the urine of NPC and control patients were monitored is useful for differentiating control patients from the NPC patients.

Significance to Biomedical Research and the Program of the Institute: Sero-epidemiological surveys have demonstrated a relationship between EBV and cancer. These studies will provide information as to whether EBV plays a role in NPC and if the association of the EBV genome in the epithelial cells of the tumor is important for the induction of the tumor.

Proposed Course: The basic research portion of the contract terminated on March 31, 1981. The nonhuman primates under test will be maintained and observed for 12 months.

Date Contract Initiated: March 29, 1978

Current Annual Level: $6,000

SAINT LOUIS UNIVERSITY (NO1-CP4-3359)

Title: Search for Viral-Specific Genetic Material in Human Cancers and Studies on the Mechanism of Oncogenesis by DNA Tumor Viruses.

Contractor's Project Director: Dr. Maurice Green

Project Officer (NCI): Dr. Maurice L. Guss

Objectives: This research program is aimed to achieve increased understanding of the mechanism of cell transformation by DNA tumor viruses and to apply new information on viral carcinogenesis and molecular biology of human cells directly to the problems of human cancer.

Major Findings: Human cancers representing 90 per cent of the cancer incidence (15 categories of human cancer) in the United States have been analyzed for transforming genes of five different groups of human adenoviruses (Ads) representing Ads 1-31. All cancers so far have been negative; thus, human Ads as causes of most human cancers may be excluded. To date, cancers representing 50-60 per cent of cancer incidence have been negative for sequences of human BK virus. Cancers representing 50 per cent incidence were also negative for sequences of two distinct human papillomaviruses (HPV), HPV-1 and HPV-2. For these cancer studies the genomes of six different JCV isolates, and of five different human papillomaviruses, HPV-1 to HPV-5, have been cloned.

Over 2,500 human cancer specimens, and extracted nucleic acids from over 700 of these, have been collected. A partial library of cancer DNA Southern blots, in which cancer DNAs are transferred to DBM-paper or DPT-paper, has been prepared. Since the DNA is covalently bound to the paper, the blot can be assayed repeatedly using different probes. Hybridization is performed by the newly developed dextran sulfate procedure which can detect ~0.2 copies per tumor cell of viral gene sequences of 1×10^6 daltons.

Significance to Biomedical Research and to the Program of the Institute: This is a systematic study using the most sensitive techniques available to probe for evidence of an association between members of the adenovirus and papovavirus groups and neoplasms occurring in humans.

Proposed Course: This contract terminated on March 31, 1981.

Date Contract Initiated: March 20, 1967

Current Annual Level: No Funds in FY81

SUMMARY REPORT

RNA VIRUS STUDIES (I)

The studies in the RNA Virus (I) component supported by the contract mechanism are concerned with research on viruses of mouse, hamster, cat, bovine or primate origin containing a ribonucleic acid core which are known or suspected to be involved in the induction of malignant transformation of animal and human cells. The approach used for most of the investigations has been to study several model systems for evidence of viral carcinogenesis. Such animal studies are necessary for developing reagents, techniques and new approaches that might be applicable to studies concerning the etiology and control of human cancer. These studies can be divided into three major categories: (1) detection of retroviral information in human tumors, (2) studies related to cancer prophylaxis, and (3) the role of type B and type C RNA viruses in differentiation, transformation and carcinogenesis.

Detection of Retroviral Information in Human Tissues: One approach is the construction of hybridization probes to detect homologous type C viral sequences in human cells. Immunologic evidence suggests that certain proteins produced by the Hodgkin's lymphoma SU-DHL-1 cell line are antigenically related to products coded for by viruses of the GaLV/SSAV groups; therefore, this group of viruses has been used in most hybridization studies. In preparation for these studies the gibbon type C (GaLV) viral genome has been cloned in a lambda phage vector and mapped with restriction endonucleases. Several fragments have been subcloned in the pBR322 plasmid vector and labeled by nick-translation for use as a molecular hybridization probe. Further characterization of the probe is ongoing.

The reactivity of a monoclonal antibody [(3D7(2B9)] which detects a 28,000 dalton protein p28 in the SU-DHL-1 virus and also crossreacts with the p28 of SSV-1/SSAV has been compared with that of a heterologous rabbit antibody which has previously been shown to be monospecific for the p28 of SSV-1/SSAV. In competition radioimmunoassays, SSAV-1/SSAV preparations competed with the SU-DHL-1 p28 for binding to the 3D7(2B9) antibody, but with a different slope than that observed with the homologous antigen. This indicates that the SSV-1/SSAV antigenic site with which this antibody crossreacts is only partly homologous with the antigenic site on the SU-DHL-1 viral p28. Also, the monospecific heterologous antiserum to SSV-1/SSAV p28 precipitates two proteins in the 60-65,000 dalton range which are translated from mRNA of approximately 30-35S in SSV-1/SSAV infected cells, whereas the 3D7(2B9) monoclonal antibody to the SU-DHL-1 viral p28 precipitates only the 65,000 dalton translation product of SSV-1/SSAV viral RNA.

Using a method for demonstrating primer tRNA binding to poly(A)RNA molecules, it is now possible to demonstrate various specific poly(A)RNA molecules in cultured human cells. Initial studies indicated that a human breast cancer cell line (ALAB) contained a unique primer tRNA binding an RNA molecule of 24S size. This molecule is present in all human breast cancer epithelial cell lines examined, but not in epithelial cell lines of other human cancers, nor in the fibroblast cells derived from human breast cancers. It apparently binds selective tRNA species, the identity of which is being investigated. In the reverse transcription reaction, in which avian myeloblastosis reverse transcriptase is added to a mixture of poly(A)RNA preparations of the cell and total tRNA's, a copy

DNA of about 140 base lengths linked to a 70 base RNA primer can be demonstrated. Preliminary results suggest that content of this RNA molecule in the ALAB cells may be stimulated by hydrocortisone treatment.

Molecular binding using plasmids and the Charon series of λ phage and in vitro translation using the rabbit reticulocyte system have been initiated to characterize the 24S RNA molecule of human breast cancer cells. Employing a molecular clone of mouse rRNA gene, the sites of proline tRNA binding at the 3' portion of mouse 28S rRNA have been located. This shows that selective tRNA·rRNA interaction phenomenon (in an in vitro hybridization reaction) is not due to nonspecific binding. Because of the vast sequence heterogeneity and possible marked evolutional changes of the large terminal repeats (LTR) of retroviral DNA, it is apparent that the primer tRNA approach may be useful for the detection and isolation of this class of transposable gene elements in human cells.

Studies are continuing to define an antigen in human mammary neoplasia which is immunologically related to the major envelope glycoprotein (gp52) of murine mammary tumor virus and determine the significance of this crossreactivity. Significant progress has been made in the purification of the gp52 related antigen from clones 8, 10 and 11 of the 47D human breast tumor cell line. The particles containing the relevant antigen banded at 1.16 to 1.19 g/ml and the yield was 0.2-0.5 mg of particles/liter of medium. Certain characteristics of these particles have been established including incorporation of radioactive uridine, leucine and glucosamine. The uridine incorporated material can be extracted by phenolization as an RNA molecule which bands at 1.65 in a cesium sulfate density gradient and sediments as a 70S molecule in an SDS sucrose gradient. It further appears that the yield of the 70S RNA with clones 10 and 11 increases as the estradiol concentration is increased from 10^{-10} M to 10^{-6} M. Appropriate examination of the particles shows the presence of reverse transcriptase in all particle preparations from the three clones examined. An endogenous reaction with radioactive deoxytriphosphates leads to incorporation into an acid-insoluble DNA which bands at a density of 1.45 in cesium sulfate gradient. However, if the total nucleic acid is run, it is found that a portion of the DNA sediments with the 70S RNA molecule and the DNA is then found in the RNA density region of a cesium sulfate density gradient. Pretreatment with either alkali or with ribonuclease shifts these counts to the DNA region of the density gradient.

Although clones 8, 10 and 11 of the tumor cell line 470 appeared to possess characteristics suitable for the production of human antigen, it was noted that the antigenic activity of the clone 10 particles split into two peaks in glucose gradients, one banding at 1.16 to 1.19 g/ml and another between 1.20 and 1.24 g/ml. The latter is usually the density region in which cores of C and B type particles are found, and it seemed possible that this might be the case here. After extensive testing in glucose gradients it was found that all of the antigen cross-reacting with the human gp52 from all three cell line clones shifted in a similar way to the 1.20-1.24 g/ml region.

Another method for greatly enriching the relevant human antigen can be carried out with whole particles using a denaturing agarose column. The results show that one particular fraction (no. 14) uniformly contains the antigenically active material and exhibits the usual heterologous interaction. The column chromatography enriches the content of the active antigen by a factor of 500 times over the material found in the particle density region (1.16-1.19 g/ml) of an isopycnic separation in a sucrose gradient.

Studies Related to Cancer Prophylaxis: Two projects address immunoprevention of cancer in the cat. In the first study, the efficacy of feline leukemia soluble tumor cell antigen vaccine (STAV) has been confirmed in conventional cats (non-SPF). This vaccine was tested in a large multi-cat household. All vaccinates produced antibody to the feline oncornavirus cell membrane antigen (FOCMA) and the degree of immunoprotection was equivalent to that reported earlier with SPF cats ($\tilde{\geq}$ 80%). Recently, a one-way mixed lymphocyte reaction (MLR) test for cats was developed to measure suppressor cell function. Studies indicate the cats infected with FeLV exhibit a striking loss of suppressor cells. The immunosuppressive retroviral protein (FeLV p15E) abrogates the MLR reaction. RD-114 virus p20 also interferes with lymphocyte functions including lymphocyte capping, lymphocyte blast transformation and MLR.

In another study, cats vaccinated with CCC81(S^+L^-) cells superinfected with F422 FeLV developed a mild transient viremia, but subsequently were strongly refractory to oncogenic virus challenge with Snyder-Thielen feline sarcoma virus (FeSV)/FeLV. Normal CCC81 cells appeared to protect against FeSV but not FeLV, but superinfected CCC81 cells provided resistance to both FeSV and FeLV. Some difference was noted between the protective effect of low and high passage F422 FeLV when used to infect CCC81 cells; low passage virus produced the higher level of protection.

Cats vaccinated with FeSV transformed, FOCMA positive, non-producer mink cells (64F3CL7) were no more resistant to FeSV/FeLV challenge than were cats vaccinated with FOCMA negative normal mink cells (CCL64). Attempts to increase the antigenicity of FeLV by binding to xenogeneic red cells or to sepharose 4B did not result in vaccines with marked protective effects against oncogenic virus challenge.

Studies using purified viral structural proteins and the corresponding antisera for immunological control of viral disease have been performed in mice. AKR mice were treated with heterologous anti-gp71 antibodies under various conditions in order to establish the optimal criteria for effective suppression of leukemia development. The strongest effect was observed when mice were treated at birth; and when this regimen was used, prior treatment of the mothers did not provide additional protection. If treatment was delayed until day 3, the beneficial effect of the serum diminished sharply, emphasizing the presence of a narrow window very early in the life of the AKR mouse when antibody must be present in order to have an effect on subsequent leukemia development. A number of parameters were examined in the experimental mice, and as in a previous study; suppression of leukemia, which occurred in 68% of the animals, correlated with the elimination of viremia and appearance of natural anti-viral antibodies. Interestingly, the results suggest that antibody therapy is primarily effective against the thymic form of the disease.

The availability of non-viremic, antibody-positive animals made possible the opportunity to examine if these characteristics could be transmitted to the offspring. From selected mating crosses, both F1 and F2 generations of AKR mice which possessed high titers of anti-viral antibodies and were non-viremic at 21-28 weeks of age were successfully derived. It appeared that a maternal effect may be responsible for this phenomenon.

A survey of MuLV-leukemia accelerating activity in AKR mice was the finding that a nonleukemogenic dualtropic MuLV isolated from Moloney MuLV, designated SMX-1, actually inhibited the development of spontaneous (as well as MuLV-accelerated)

leukemia. Intrathymic injection of SMX-1 resulted in marked reduction in the incidence of spontaneous leukemia; 35% at 1 year as compared to an incidence of 98% in control AKR mice. SMX-1 had no inhibitory activity when injected by the IP route but was effective after IV injection. Protection appeared maximal when SMX-1 was injected into 1 or 2-month-old mice. Less protection was seen when mice were injected at 110 days and no protection if injection was delayed until 6 months of age.

The usefulness of the KiMSV tranformation assay in identifying individuals at risk in dominant cancer gene families is still under study. Growth characteristics and susceptibility to viral transformation were compared in cultured skin fibroblasts from patients with Gardner's syndrome (GS), those with familial polyposis coli (FP), asymptomatic family members, and unrelated controls. Compared to cells from unrelated controls, cells from 4 of 5 GS patients were tranformed by Kirsten murine sarcoma virus at 100- to 1000-fold increased efficiency. The transformation efficiencies of fibroblasts from 2 of 3 FP patients were 10- to 100-fold greater than those of unrelated controls. However, because the fibroblasts from some of the young asymptomatic GS and FP family members also transformed at higher efficiency than did cells from unrelated controls, long-term observation of these families is required. This would determine whether or not these members develop clinical manifestations of GS or FP and thus establish the specificity of this assay for detection of individuals bearing the mutant gene. Compared to fibroblasts from unrelated controls, GS and FP fibroblasts showed a two-fold to three-fold increased saturation density and plating efficiency, but this difference was not noted with fibroblasts from most of the asymptomatic family members. The plating efficiency of GS and FP fibroblasts was not enhanced by treatment with the tumor promoter TPA, nor were the treated cells morphologically altered.

Role of Type C Virus Expression in Differentiation, Transformation and Carcinogenesis: The envelope protein of the virion as well as the product of the sarc gene (transforming protein) are under study in the mouse and the cat. The envelope protein may be important because specific antibody to this protein may prevent development of cancer; the transforming protein because it is thought to be a key substance required for malignant transformation. Several studies in mice are attempting to identify the cooperating receptor molecule involved in virus-cell interaction. In vitro studies have demonstrated the presence of a protein fraction, termed BPgp70 for its property as Binding Protein for gp70, which has two properties in addition to its ability to bind reversibly to gp70 (inhibiting its binding to 3T3 cells). These properties are the alteration of morphology of uninfected 3T3 cells or 3T3 cells infected with MCF viruses (but not 3T3 cells infected with ecotropic viruses) and the inhibition of DNA synthesis in uninfected 3T3 cells. BPgp70 was subjected to further purification by preparative gel filtration chromatography and gel electrophoresis. The gp70 binding inhibiting activity and properties noted above cofractionated by these procedures. The most active BPgp70 fractions have been tested for homogeneity by sequencing analysis, which yielded no defined sequence pattern and indicated a lack of homogeneity in the preparation.

BPgp70 has been treated with a number of reagents, including enzymes, site specific reagents, and protein denaturants to test for any similarity in chemical sensitivities between the activities which block gp70 binding and those that induce morphological alterations in cells. These activities are nondialyzable, and not sensitive to heating for 10 min at 100°. Treatment of BPgp70 with trypsin, chymotrypsin, glycosidases, imidates, acetic anhydride, guanidine, or urea did

not inhibit either activity. However, BPgp70 was inactivated by treatment with acid or base, and the inactivation rates for the binding and cellular activities were similar.

Two types of cell mediated cytolytic activity were identified in AKR spleen cells obtained from animals which have been previously primed with lethally irradiated tumor cells. The first does not require in vitro stimulation with tumor cells and is also found in normal AKR mice. It appears refractory to the effects of anti-theta and complement, antigen-antibody complexes, and is not affected by the removal of adherent cells. These results imply such reactivity is mediated by natural killers. The second type of activity requires in vitro challenge with irradiated tumor cells.

Dual-tropic MuLV, but not ecotropic or xenotropic MuLV, are capable of amplifying expression of MuLV-related antigens on thymocytes (A^+ trait) and of accelerating leukemia development (L^+ trait). Both A^+L^- and A^+L^+ dualtropic MuLV isolates were identifed, indicating that these 2 activities of antigen amplification and leukemia acceleration represent separable viral phenotypes. Although dual tropic viruses can be classified serologically into 3 distinct phenotypes, no serological markers have been found to distinguish A^+L^- from A^+L^+ MuLV. A single infectious unit of A^+L^+ MuLV (measured by in vitro assay) was capable of antigen amplification in vivo and analysis of the surface antigenic phenotype of amplified thymocytes or accelerated leukemias indicates direct coding by the input virus rather than by new MuLV species resulting from additional recombinational events after infection and leukemic transformation.

A 55,000 dalton rat cell membrane glycoprotein (gp55) possibly related to the transformation process has been purified to homogeneity and characterized. This protein was originally identified in preparations of a defective pseudotype of the Kirsten sarcoma virus and shown to be present in several rodent retrovirus particles. The gp55 was purified from this defective virus by conconavalin A and heparin affinity chromatography as well as by preparative SDS-gel electrophoresis. The ^{125}I-labeled gp55 was precipitated by antisera against rodent retroviruses, but not by monospecific antisera against purified type C virus structural proteins, thus indicating that gp55 was retrovirus associated, but unrelated to known retrovirus structural proteins. Competition radioimmunoassay indicated that the gp55 is a cell membrane glycoprotein associated in high concentrations with retroviruses.

Studies in the cat have shown that spontaneous and FeSV-induced tumor cells were examined after explantation in vitro for expression of the FeSV-related "gag-x", p110, polyproteins. Those transformation-specific proteins related to each strain of FeSV were regularly found in cells from tumors induced by that strain but no "gag-x" proteins were found in spontaneous tumors that were unrelated to FeLV-FeSV. The proteins were expressed in cat cells that produced FeLV as well as in non-feline nonproducer cells (mink, rat). It was determined that cats could respond to the "X" portion of "gag-x" and that such "X" specific sera give a typical FOCMA type reaction on lymphoid cells. Murine and cat cells were transfected with restricted and unrestricted FeSV proviral DNA and transformed with the induction of ST-FeSV "gag-x". Some transfection studies were done with DNA from feline lymphomas and an unusual "strain" of FeLV was isolated based on host-range criteria. FeSV was used to induce nonproducer tumors in rats, and such tumor cells were found to contain "gag-x". Using the fluorescence activated cell sorter, it was demonstrated that all of the FeLV virion structural proteins were expressed at the cytoplasmic membrane of cultured producer lymphoma cells. Several candidate

monoclonal antibodies were examined for activity to "gag-x". Nonviremic cats that were naturally exposed to FeLV and had high FOCMA antibody titers were found to maintain those high antibody titers in the absence of re-infection, suggesting the possibility that FOCMA positive cells may persist in some virus-negative animals.

Type B Viral Expression in Differentiation, Transformation and Carcinogenesis: At the present time, breast cancer is known to be caused by a virus in only one animal species, the mouse. The mouse mammary tumor virus (MuMTV) mouse model system has been utilized for a variety of studies, including MuMTV replication and expression, MuMTV biology and characterization, and the effect of viral-chemical interaction on tumor induction. The expression and function of each MuMTV is influenced by many factors (genetic, hormonal, environmental) operating within and on each mouse. Several projects are investigating the effects of environmental factors on endogenous MuMTV expression in low mammary strains of mice. Tumors were induced in virgin female animals by treatment with chemical carcinogens 7,12-dimethylbenz(a)anthracene (DMBA) or urethan, with or without prolonged hormonal stimulation, or by X-irradiation. Concomitant hormonal stimulation resulted in increased tumor incidences over those induced by chemical carcinogen treatment alone. The frequency of tumor induction by irradiation alone or in combination with urethan or prolactin stimulation was very low.

MuMTV RNA expression in the mammary tumors was assayed by nucleic acid hybridization. Tumors which contained detectable viral transcripts exhibited only low levels of RNA which did not appear to represent the accumulation of RNA sequences homologous to the entire MuMTV genome; detectable synthesis of MuMTV structural proteins occurred in only one tumor.

Viral RNA-positive tumors were generally associated with a longer latent period. MuMTV RNA expression occurred in both histological types of tumors, mammary adenocarcinomas and adenoacanthomas. It does not appear that expression of the endogenous MuMTV genome is required for maintenance of all mammary tumors in BALB/c mice, although partial genome expression undetectable by the methods employed cannot be ruled out.

The neoplastic progression of the murine mammary gland involves an intermediary stage, the hyperplastic alveolar nodule (HAN), which can be morphologically visualized as lobular alveolar tissue in a non-pregnant, non-lactating host. The HAN can be surgically removed and transplanted into the cleared fat pad of isologous hosts. The resultant growth of the HAN is the hyperplastic outgrowth (HOG), which is delineated by the boundaries of the fat pad, and which has a higher tumor risk than normal mammary epithelium. This transplantation technique allows investigators to experimentally manipulate the HAN and to amplify the number of cells in the HAN for biochemical studies. By serially transplanting the preneoplastic HAN in mammary fat pads, HOG have been established in strains C3H, GR, BALB/cNIV, and BALB/cC3H. New outgrowth lines are in the process of being developed from three low tumor incidence mouse strains [C3H/StWi, (C57Bl x DBAf)F_1, and BALB/cVo]. Six different HAN lines have been established from DMBA-treated or pituitary-isograft-bearing BALB/cVo animals, which express the putative endogenous MuMTV. All the lines are tumorigenic. Preliminary results indicate the outgrowth lines can grow and are tumorigenic in BALB/c animals, as well as in the parental BALB/cVo animals. All the HAN lines and most of the tumors which arose from the HAN lines expressed MuMTV antigens. Restriction maps of the five HOGs derived from BALB/cfC3H strain demonstrate that all contain the

2.5×10^6 dalton Pst I fragment indicative of the exogenous C3H MuMTV. All HOGs have their own unique restriction patterns and these patterns appear to be stable during the fourth and eighth through eleventh transplant generations. Some tumors which arise from these HOGs contain additional restriction bands. However, all tumors contain the restriction pattern observed in their respective outgrowths.

Antisera are being prepared in rabbits against intact and disrupted (C3H)MuMTV as well as MuMTV purified polypeptides. The antiserum made against disrupted (C3H)MuMTV is able to recognize virus-specific proteins induced by the BALB/c virus. The molecular weights of the (BALB/c)MuMTV proteins correspond to those of the standard C3H virus. Milk fat globule immunogen prepared from BALB/c mouse milk was used to raise mammary epithelial cell-specific antibody.

In vitro translation of RNA's from D2 HAN, D2 tumors and BALB/cfC3H tumors resulted in the synthesis of p28 while BALB/cfC3H tumor mRNA translated into similar amounts of the core and envelope polypeptides. The 25S RNA fraction from the BALB/cfC3H tumors directed the synthesis of gp52, while 35S and 13S did not. Synthesis of gp52 was not detected in the translation products of 25S RNA from either D2 HAN or D2 tumors. It was concluded that either endogenous MuMTV in BALB/cfC3H D2 tissues expresses a defective envelope mRNA, specific translation factors are required for its translation, or its translation product is antigenically unrelated to MuMTV-S envelope and escapes immunodetection. An antigenically altered endogenous MuMTV envelope gene product, by analogy to certain murine type C recombinant transforming viruses, could have important implications regarding the oncogenic potential of endogenous MuMTV in mammary epithelium.

RNA VIRUS STUDIES (I)

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BAYLOR COLLEGE OF MEDICINE (NO1-CP8-1006)

Title: Effects of Environmental Factors on Endogenous MuMTV Expression in Low Mammary Strains of Mice

Contractor's Project Director: Dr. Janet S. Butel

Project Officer (NCI): Dr. Yoshio Teramoto

Objectives: To induce MuMTV expression and mammary tumor development in mice having a low incidence of spontaneous mammary neoplasms by the use of chemical carcinogens and x-irradiation.

Major Findings: Tumors were induced in virgin female animals by treatment with chemical carcinogens 7,12-dimethylbenz(α)anthracene (DMBA) or urethan, with or without prolonged hormonal stimulation, or by x-irradiation. Concomitant hormonal stimulation resulted in increased tumor incidences over those induced by chemical carcinogen treatment alone. The frequency of tumor induction by irradiation alone or in combination with urethan or prolactin stimulation was very low.

MuMTV RNA expression in the mammary tumors was assayed by nucleic acid hybridization. Depending upon the treatment group, from 0-83% of tumors contained detectable levels of MuMTV RNA ($\geq$0.0005% of total cellular RNA). Tumors which contained detectable viral transcripts exhibited only low levels of RNA which did not appear to represent the accumulation of RNA sequences homologous to the entire MuMTV genome; detectable syntnesis of MuMTV structural proteins occurred in only one tumor.

Viral RNA-positive tumors were generally associated with a longer latent period. MuMTV RNA expression occurred in both histological types of tumors, mammary adenocarcinomas and adenoacanthomas. It does not appear that expression of the endogenous MuMTV genome is required for maintenance of all mammary tumors in BALB/c mice, although partial genome expression undetectable by the methods employed cannot be ruled out.

Significance to Biomedical Research and the Program of the Institute:
It is well known that hormones, chemicals and ionizing irradiation may enhance the development of cancer. These same factors also seem to influence expression of oncogenic RNA tumor viruses and host cell transformation. Knowledge of the extrinsic factors which can affect viral gene activation may provide information leading to the control of neoplasia.

Proposed Course: This contract terminated January 12, 1981.

Date Contract Initiated: January 13, 1978

Current Annual Level: No funding in FY 81

BAYLOR COLLEGE OF MEDICINE (NO1-CP9-1020)

Title: Induction and Control of MuMTV Expression in Mouse Mammary Preneoplastic Tissues

Contractor's Project Director: Dr. Janet S. Butel

Project Officer (NCI): Dr. Yoshio Teramoto

Objectives: To investigate the induction and control of MuMTV expression in mouse mammary preneoplastic tissues as a model system for developing concepts, techniques and reagents which can be applied to precancerous human mammary tissues.

Major Findings: New outgrowth lines are in the process of being developed from three low tumor incidence mouse strains [C3H/StWi, (C57Bl x DBAf)F_1, and BALB/cVo]. Six different hyperplastic alveolar nodule (HAN) lines have been established from DMBA-treated or pituitary-isograft-bearing BALB/cVo animals, which express the putative endogenous MuMTV. All the lines are tumorigenic. Preliminary results indicate the outgrowth lines can grow and are tumorigenic in BALB/c animals, as well as in the parental BALB/cVo animals. All the HAN lines and most of the tumors which arose from the HAN lines expressed MuMTV antigens.

The C1-S1 tissue culture cell line derived from the BALB/c D-1 HAN line has been obtained from H. Hosick. Many cells in culture express MuMTV antigens. The cell line appears to undergo morphological changes (differentiation?) when cultured in suspension in collagen gels.

Antisera are being prepared in rabbits against intact and disrupted (C3H)MuMTV as well as MuMTV purified polypeptides. The antiserum made against disrupted (C3H)MuMTV is able to recognize virus-specific proteins induced by the BALB/c virus. The molecular weights of the (BALB/c)MuMTV proteins correspond to those of the standard C3H virus. Milk fat globule immunogen prepared from BALB/c mouse milk was used to raise mammary epithelial cell-specific antibody. Additional studies must be performed before it is established whether a usable reagent has been obtained.

Significance to Biomedical Research and the Program of the Institute: Recent reports indicate that small atypical lesions, similar to the hyperplastic nodules in murine mammary cancer, exist in the dysplastic and carcinoma-in-situ cells of the human breast and that hyperplastic regions of the human mammary gland appear to contain an antigen that demonstrates crossreactivity with the glycoprotein (gp52) of MuMTV, therefore, it would be worthwhile to investigate the induction and control of MuMTV expression in mouse mammary preneoplastic tissues as a model system which can then be applied to an understanding of precancerous human mammary tissues.

Proposed Course: Efforts will continue to: conduct virological investigations to propagate sufficient quantities of HAN tissues (or outgrowth lines) in selected MTV-positive and MTV-negative BALB/c and other mouse strains; detect and quantitate levels of MuMTV expression in HAN tissues of varying tumorigenicities in selected mouse strains; and attempt to alter virus expression in HAN tissues with immunological reagents, chemicals and/or hormones in efforts to define

regulatory controls which may determine functions of viral components during mammary tumorigenesis.

Date Contract Initiated: September 1, 1979

Current Annual Level: $144,800

CALIFORNIA, UNIVERSITY OF, DAVIS (NO1-CP3-3242)

Title: Comparative Leukemia and Sarcoma Viral Studies

Contractor's Project Director: Dr. Thomas G. Kawakami

Project Officer (NCI): Dr. Garrett V. Keefer

Objectives: To detect, isolate and characterize type C viruses from spontaneous malignancies of primates and humans.

Major Findings: The contractor has been monitoring four experimental animals that are viremia negative and exhibiting elevated leukocyte counts of 12,000-19,000 with a predominance of mature granulocytes. To date these animals have not developed chronic myelocytic leukemia.

In the entire colony of 56 gibbons, 32 are free of gibbon ape leukemia virus (GaLV) infection and 24 are infected with GaLV. Of the 24 infected animals, 20 have persistent antibody while 4 are viremic. Although several breeders are antibody positive for GaLV, all their offspring have been born free of detectable GaLV infection. Only viremic animals appear to pass their infection to their offspring. While numerous gibbons are antibody positive, it still is not known whether any of these animals will convert to persistent viremia. The conversion from persistent antibody to viremia must be a rare event because only one male naturally infected with GaLV has had occasional viremia.

The last six months of the contract were devoted to finding appropriate homes for these animals in various zoos around the United States.

Significance to Biomedical Research and the Program of the Institute: This gibbon colony offers a unique opportunity for the study of type C virus-associated leukemias spontaneously occurring in an ape. The virus materials obtained may be important as probes for the detection of crossreactive components in human cancer tissues. Comparative study of normal animals with those that develop disease provides opportunity to obtain some insight into host factors related to expression of disease.

Proposed Course: This contract terminated June 30, 1981.

Date Contract Initiated: November 1, 1969

Current Annual Level: No funding in FY 81

CALIFORNIA, UNIVERSITY OF, DAVIS (NO1-CPO-1008)

Title: Induction and Control of MuMTV Expression in Mouse Mammary Preneoplastic Tissues

Contractor's Project Director: Dr. Robert D. Cardiff

Project Officer (NCI): Dr. David Colcher

Objectives: To investigate the induction and control of MuMTV expression in mouse mammary preneoplastic tissues as a model system for developing concepts, techniques and reagents which can be applied to precancerous human mammary tissues.

Major Findings: Hyperplastic outgrowths (HOG) have been established in strains C3H, GR, BALB/cNIV, and BALB/cC3H. They are currently in transplant generations 2, 2, 4, and 2 respectively. These HOGs were derived by transplantation of either an individual hyperplastic alveolar nodule (HAN), or segments of HANs dissected into halves, fourths or eighths. Determination of their virological, phenotypical and tumorigenic stability will be determined.

Restriction maps of the five HOGs from BALB/c C3H mice demonstrate that all contain the 2.5×10^6 dalton Pst I fragment indicative of the exogenous C3H MuMTV. All HOGs have their own unique restriction patterns and these patterns appear to be stable during the fourth and eighth through eleventh transplant generations. Some tumors which arise from these HOGs contain additional restriction bands. However, all tumors contain the restriction pattern observed in their respective outgrowths.

Monoclonal antibody production against MuMTV using the hybridoma technique has been initiated.

Breeding colonies of several mouse strains have been established.

Significance to Biomedical Research and the Program of the Institute:
It has been reported that small atypical lesions, similar to the hyperplastic nodules in murine mammary cancer, exist in the dysplastic and carcinoma-in-situ cells of the human breast and that hyperplastic regions of the human mammary gland appear to contain an antigen that demonstrates crossreactivity with the glycoprotein (gp52) of MuMTV. Therefore, it would be worthwhile to investigate the induction and control of MuMTV expression in mouse mammary preneoplastic tissues as a model system which can then be applied to a better understanding of precancerous human mammary tissues.

Proposed Course: Efforts will continue to: conduct virological investigations to propagate sufficient quantities of HAN tissues (or outgrowth lines) in selected MTVpositive and MTVnegative BALB/c and other mouse strains; detect and quantitate levels of MuMTV expressions in HAN tissues of varying tumorigenicities in selected mouse strains; and attempt to alter virus expression in HAN tissues with immunological reagents, chemicals and/or hormones in efforts to define regulatory controls which may determine functions of viral components during mammary tumorigenesis.

Date Contract Initiated: October 1, 1979

Current Annual Level: $177,590

CALIFORNIA, UNIVERSITY OF, LOS ANGELES (NO1-CP9-1010)

Title: Identification of Cell Surface Receptor for Oncornavirus gp70 on Murine Fibroblasts

Contractor's Project Director: Dr. C. Fred Fox

Project Officer (NCI): Dr. Garrett V. Keefer

Objectives: To obtain sufficient quantities of purified gp70 receptor for biochemical characterization and production of specific antiserum.

Major Findings: Studies on a protein fraction which was isolated from the culture fluid of BALB/c 3T3 cells and interacts avidly with gp70 from Rauscher leukemia virus is continuing. This protein fraction, termed BPgp70 for its property as Binding Protein for gp70, has two activities in addition to its ability to bind reversibly to gp70, inhibiting its binding to 3T3 cells. These properties are: (i) alteration of morphology of uninfected 3T3 cells or 3T3 cells infected with MCF viruses, but not 3T3 cells infected with ecotropic viruses and (ii) inhibition of DNA synthesis in uninfected 3T3 cells. BPgp70 was subjected to further purification by preparative gel filtration chromatography and gel electrophoresis. The gp70 binding inhibiting activity and properties (i) and (ii) above cofractionated by these procedures. The most active BPgp70 fractions have been tested for homogeneity by sequencing analysis, which yielded no defined sequence pattern and indicated lack of homogeneity in the preparation. Additional batch scale fractionations are in progress to attempt further purification.

BPgp70 has been treated with a number of reagents, including enzymes, site specific reagents, and protein denaturants to test for relatedness in chemical sensitivities of the activities which block gp70 binding and induce morphology alterations in cells. These activities are nondialyzable, and not sensitive to heating for 10 min at 100°. Treatment of BPgp70 with trypsin or chymotrypsin or with glycosidases, followed by heating to inactivate the added enzymes, resulted in no inhibition of BPgp70 activity compared with the effects of treated control solutions containing no BPgp70. Site specific reagents, including imidates or acetic anhydride, and denaturants such as guanidine or urea, likewise had no effect. BPgp70 was inactivated by treatment with acid or base, and the inactivation rates for the binding and cellular activities were similar.

The gp70 binding assay has been refined further to effect greater reproducibility in the gp70 binding assays. All radioiodination procedures currently in common use for generation of radiolabeled ligands for binding assays produce noncovalent gp70 oligomerization at gp70 concentrations in the normal assay range (1-2 nM). Preparations of gp70 rich in these oligomers are generally unsatisfactory for reproducible binding assays and are characterized by high nonspecific gp70 binding to cells. Procedures have been defined to minimize the formation of gp70 oligomers during radioiodination.

Significance to Biomedical Research and the Program of the Institute: Infection with oncornaviruses may lead to malignant transformation in many animal species; prevention of infection can thus prevent the development of malignancy. It has been determined that the gp69/71 of the virion is the virus receptor molecule; specific antibody to this protein may prevent development of cancer. However, the cooperating cellular receptor molecule has not been definitively

identified. Information relevant to the virus-cell interaction is basic to determining the complete course of events leading to malignant transformation.

Proposed Course: Purification of the binding protein for RLV gp70 (BPgp70) and its molecular and biological characterization will continue.

Date Contract Initiated: November 15, 1978

Current Annual Funding: $69,310

COLUMBIA UNIVERSITY (NO1-CP7-1016)

Title: The Diagnostic and Clinical Implications of Viral-Related Proteins in Human Cancer

Contractor's Project Director: Dr. Sol Spiegelman

Project Officer (NCI): Dr. Takis Papas

Objectives: To define an antigen in human mammary neoplasia which is immunologically-related to the major envelope glycoprotein (gp52) of murine mammary tumor virus and determine the significance of this crossreactivity.

Major Findings: Significant progress has been made in the purification of the gp52 related antigen from clones 8, 10 and 11 of the 47D cell line. The particles containing the relevant antigen bonded at 1.16 to 1.19 g/ml and in general the yield was 0.2-0.5 mg of particles/liter of medium. Certain characteristics of these particles have been established, including incorporation of radioactive uridine, leucine and glucosamine. The uridine incorporated material was extracted by phenolization as an RNA molecule which bands at 1.65 in a cesium sulfate density gradient and sediments as a 70S molecule in an SDS sucrose gradient. It further appears that the yield of the 70S RNA with clones 10 and 11 increased as the estradiol concentration was increased from 10^{-10} M to 10^{-6} M. Appropriate examination of the particles showed the presence of reverse transcriptase in all particle preparations from the three clones examined. Carrying out an endogenous reaction with radioactive deoxytriphosphates leads to incorporation into an acid-insoluble DNA which bands at a density of 1.45 in cesium sulfate gradient. However, if the total nucleic acid is run, it was found that a portion of the DNA sediments with the 70S RNA molecule and the DNA was then found in the RNA density region of a cesium sulfate density gradient. Pretreatment with either alkali or with ribonuclease shifted these counts to the DNA region of the density gradient.

Although clones 8, 10 and 11 appeared to possess characteristics suitable for the production of human antigen, it was noted that the antigenic activity of the clone 10 particles split into two peaks in an isopycnic separation in glucose gradients, one banding at 1.16 to 1.19 g/ml and another between 1.20 and 1.24 g/ml. The latter is usually the density region in which cores of C and B type particles are found, and it seemed possible that this might be the case here. After extensive testing, it was found that all of the antigen which crossreacts with gp52 in the human particles from all three clones shifted from 1.16 to 1.19 g/ml to 1.20 to 1.24 g/ml. This means that the portion of the human particle that crossreacts with gp52 is confined principally to the core.

A method for greatly enriching the relevant antigen has been carried out with whole particles using a denaturing agarose column. The results showed that one particular fraction (no. 14) uniformly contains the antigenically active material and exhibits the usual heterologous interaction. The column chromatography enriches the content of the active antigen by a factor of 500 times over the material found in the particle density region (1.16-1.19 g/ml) of an isopycnic separation in a sucrose gradient.

The contractor has made one remarkable observation whose significance they are now actively exploring. This came in the form of some new antisera raised in New Zealand white rabbits which possess properties rather different from those exhibited by sera obtained from other rabbit strains. In particular, they found that certain of these antibody preparations stain over 90% of the breast cancers as compared with only 46% using the old serum. The contractor is quite certain that he can reproduce the production of this type of antiserum, at least in this strain of rabbit. Presently they are trying to delineate the reasons for this difference, although they were not surprised to find that antisera could be obtained which gave almost 100% positivity.

Significance To Biomedical Research and the Program of the Institute:
A systematic molecular biological study has demonstrated the presence in human cancer of particulate materials possessing characteristics unique to the known animal RNA tumor viruses. Whether the cause or the consequence of human malignancy, the presence of the tumor related particles and their uniqueness provided a novel opportunity to generate information of potentially practical importance for the diagnosis and management of breast cancer in humans. An example is the observation that an antigen found in human breast cancer crossreacts with antibody developed to the MuMTV gp52. Definite data as to its usefulness as a practical clinical tool for the diagnosis or prognosis of human breast cancer should be obtained from this contract effort.

Proposed Course: Studies will continue on the isolation, purification and physicochemical characterization of sufficient quantities of the pertinent human antigen found in the 47D cell line. Antigens from other human breast cell lines will also be studied. Comparative biochemical studies of the human antigen and MuMTV gp52 will be performed. The distribution of the gp52-related antigen in human mammary tumors as a function of geography, family history and/or ethnic groups will be determined.

Date Contract Initiated: October 29, 1969

Current Annual Level: $620,000

CORNELL UNIVERSITY (NO1-CP9-1007)

Title: Immunoprevention of Cancer in Cats

Contractor's Project Director: Dr. Fernando de Noronha

Project Officer (NCI): Dr. Garrett V. Keefer

Objectives: To determine the nature of autogenous immunity to feline leukemia virus (FeLV) and feline oncornavirus associated antigen (FOCMA) and correlate

findings with natural disease occurrence; conduct detailed studies of immune responsiveness of specific pathogen-free (SPF) cats during vaccination with FL74 cell membrane fractions and/or purified FOCMA; conduct immunosurveillance of humans exposed to FeLV; and attempt to transform feline fibroblast cells with nonviral agents.

Major Findings: A study of the antigenic crossreactivity of feline oncornavirus-associated cell membrane antigen (FOCMA) on 5 individually derived feline leukemia virus (FeLV) replicating feline leukemia cell lines was completed. The standard test system for CDA-FOCMA was used: ^{51}Cr release from cat leukemia cells in the presence of cat FOCMA-immune sera and cat complement. To determine cross-reactivity, 170 separate sera were tested on the 5 cell lines, and quantitative absorption and cold target inhibition studies on 20 representative sera were also performed. Two separate major crossreacting antigens and a series of minor non-crossreacting individual tumor antigens were detected, suggesting that reactivity to FOCMA is directed to a spectrum of antigenic determinants.

Cell mediated immune studies in cats immunized with "allogeneic" tumor cells revealed some cytotoxic reactivity. Effector cells were detected in the spleen and/or mesenteric lymph nodes and also in blood. There were indications that some cytotoxic effects were specific but others were non-specific in nature.

Cats vaccinated with CCC81(S^+L^-) cells superinfected with F422 FeLV developed a mild transient viremia, but subsequently were strongly refractory to oncogenic virus challenge with Snyder-Thielen feline sarcoma virus (FeSV)/FeLV. Normal CCC81 cells appeared to protect against FeSV but not FeLV, but superinfected cells provided resistance to both FeSV and FeLV. Some difference was noted between the protective effect of low (36) and high (α) passage F422 FeLV when used to infect CCC81 cells; low passage virus produced the higher level of protection.

Cats vaccinated with FeSV transformed, FOCMA positive, non-producer mink cells (64F3CL7) were no more resistant to FeSV/FeLV challenge than were cats vaccinated with FOCMA negative normal mink cells (CCL64). Attempts to increase the antigenicity of FeLV by binding to xenogeneic red cells or to sepharose 4B did not result in vaccines with marked protective effects against oncogenic virus challenge.

Using CCL64 cells to titrate transforming FeSV in the blood of fibrosarcoma bearing cats, marked differences were found in the appearance and circulation of FeSV and FeLV (latter measured by CCC81 indicator cells). While FeLV viremia appeared early after virus injection and remained at high titer for several weeks, FeSV was frequently not detected in blood even when cats were bearing massive and progressing fibrosarcomas.

Significance to Biomedical Research and the Program of the Institute:
In the cat, tumors induced by FeLV and FeSV express a cell surface antigen designated "feline oncornavirus-associated cell membrane antigen" (FOCMA). Analysis of anti-FOCMA titers in sera of virus-exposed cats has suggested that development of antibody directed against FOCMA may constitute an immunosurveillance defense against tumor development. Recently, FOCMA has been shown to be distinct from all known FeLV-coded structural proteins and has been demonstrated in spontaneous lymphomas of cats even in the absence of detectable levels of FeLV structural proteins. Moreover, FOCMA has been shown to be expressed in FeSV-transformed cells in the form of a precursor containing two amino terminal FeLV structural-proteins.

These findings demonstrate FOCMA to represent a transformation-specific FeSV-coded protein, and suggest that activation of cellular gene coding for a protein analogous to FOCMA may represent a general mechanism for tumor induction in the cat.

Proposed Course: Studies concerning the development of a vaccine will continue. More specifically the ELISA technique will be modified for use in the feline system to measure levels of antigen, antibody and immune complex in FeLV exposed cats; the technique is also being evaluated as a tool to monitor human sera for evidence of FeLV exposure. FOCMA expression will be evaluated in several clones of feline fibroblasts transformed by exposure to U.V. irradiation.

Date Contract Initiated: November 1, 1978

Current Annual Funding: $150,575

DUKE UNIVERSITY (NO1-CP3-3308)

Title: Expression of the RNA Tumor Virus Genome in Animal and Human Malignant Cells

Contractor's Project Director: Dr. Dani P. Bolognesi

Project Officer (NCI): Dr. Garrett V. Keefer

Objectives: (1) To study in detail the properties of structural components of RNA tumor virus particles, particularly those of mammalian RNA tumor viruses. (2) To utilize these materials for preparation of highly specific antisera which can be applied to the analysis of cells for the presence of similar virus gene products. (3) To develop appropriate antisera which can be employed for detection and identification of tumor virus activities in human malignant cells. (4) To use purified structural viral proteins and the corresponding antisera for possible immunological control of viral disease.

Major Findings: AKR mice were treated with heterologous anti-gp71 antibodies under various conditions in order to establish the optimal criteria for effective suppression of leukemia development. The strongest effect was observed when mice were treated at birth; and when this regimen was used, prior treatment of the mothers did not provide additional protection. If treatment was delayed until Day 3, the beneficial effect of the serum diminished sharply, emphasizing the presence of a narrow window very early in the life of the AKR mouse when antibody must be present in order to have an effect on subsequent leukemia development. A number of parameters were examined in the experimental mice and, as in a previous study, suppression of leukemia, which occurred in 68% of the animals, correlated with elimination of viremia and appearance of natural anti-viral antibodies. Interestingly, the results suggest that antibody therapy is primarily effective against the thymic form of the disease.

The availability of non-viremic, antibody-positive animals made possible the opportunity to determine if these characteristics could be transmitted to the offspring. From selected mating crosses, both F1 and F2 generations of AKR mice which possessed high titers of anti-viral antibodies and were non-viremic at 21-28 weeks of age were successfully derived. It appeared that a maternal effect

may be responsible for this phenomenon. The implications of these findings in relation to the development of AKR leukemogenesis are under study.

Significance to Biomedical Research and the Program of the Institute: Although human leukemias or other tumors are not known to be associated with replicating RNA tumor viruses, one cannot exclude the possibility that virus genes exist in human cancer cells and are expressed as discrete antigens on the cell surface in a fashion similar to that in animal cells. This study has shown that, if indeed this does occur, unequivocable detection of the viral antigens on human cells may be an exceedingly difficult task to accomplish. Even so, there is considerable evidence that many other aspects, particularly the immunological consequences of animal and human leukemias, are distinctly related. Therefore, an understanding of the immunological mechanisms in the animal models which are of key importance for host defense, coupled with protocols to artifically stimulate those leading to effective prevention and control of the disease, is of value to reach a better understanding of related events in human cancer.

Proposed Course: This contractor will continue to study effective serum therapy against AKR leukemia.

Date Contract Initiated: March 1, 1973

Current Annual Funding: $189,000

ENERGY, DEPARTMENT OF (YO1-CP6-0500)

Title: NCI-ERDA Viral Carcinogenesis Program: Regulation of Gene Expression

Contractor's Project Director: Dr. Wen K. Yang

Project Officer (NCI): Dr. Charles S. Sherr

Objectives: To study the regulation of tumor virus expression, focusing on molecular mechanisms in induction and repression of tumor virus genomes. Emphasis will be placed on defining the low molecular weight RNA components of viruses and their relation to cellular RNAs.

Major Findings: Genetic crosses have been carried out between an RFM mouse strain, which restrict formation of both closed circular (form I) and linear (form III) viral DNA by B-tropic viruses, and BALB/c mouse strain, which restrict formation of form I DNA but not that of form III DNA by N-tropic viruses. Results of experiments using embryo cultures from two sets of F_2 generations and one backcross generation demonstrated segregation patterns, thereby suggesting that depression of form III (linear) DNA formation by B-tropic virus in RFM cells is specifically due to an Fv-1^n effect. Viral DNA formation was examined under three other restriction conditions, i.e., restriction of RFM cells on an N-tropic virus isolate of RFM endogenous origin, decreased susceptibility of late-passage SC-1 cells to N- and B-tropic viruses, and the Akvr gene found in the Lake Casita wild mice. Marked depression of linear DNA formation by the restricted virus was demonstrated in all three instances.

Reagents for the molecular cloning of DNA by using the charon series of λ phage and the pBR 322 plasmid system have been established. Conditions for cloning

the unintegrated retroviral DNA were investigated. Employing a Hind III cut of the closed circular viral DNA duplexes, several clones of Gross N-tropic, WN1802N, WN1802B, RFM endogenous N-tropic strains of ecotropic murine type C retroviruses have been isolated. One clone (Gross N100) contains full length viral genome with two large terminal repeats (LTR), whereas all other clones are either full length with one LTR or of subgenomic sizes. Since most λ clones grew poorly, DNA inserts were subsequently introduced into pBR 322 to obtain large quantities for further characterization. Cloning of linear viral DNAs isolated from Fv-1 restricted infection is in progress.

Significance to Biomedical Research and the Program of the Institute: This project is focused on molecular mechanisms involved in viral carcinogenesis. The problem is being investigated in terms of enzymology, immunology, cell biology, and control of gene expression primarily in the mouse leukemia system. The findings are being carried over into work with human tumor cells in an attempt to understand, and ultimately deal with, the problem of cancer in man.

Proposed Course: This contract terminated September 30, 1981.

Date Contract Initiated: July 1, 1973

Current Annual Funding: $225,730

ENERGY, DEPARTMENT OF (YO1-CP9-0503)

Title: Retroviral Genetic Expression in Human Cancers: Analysis by Primer tRNA Binding Approach

Contractor's Project Director: Dr. Wen K. Yang

Project Officer (NCI): Dr. Charles J. Sherr

Objectives: (1) Isolation of poly(A) RNAs from various human normal and cancer cells; quantitative and qualitative comparison of their capacity to bind selective tRNA species; assessment of the primer property of the bound tRNAs by reverse transcription reaction; and determination of whether different types of human cancers contain poly(A) RNA of different tRNA binding specificity. (2) Structure analysis of tRNA poly(A) RNA binding nucleotide sequences, as well as reversely transcribable 5' end sequences of the tRNA-binding site in the poly(A)RNA, by employing nucleotide sequence determination and molecular hybridization. (3) Purification of the specific poly(A)RNAs by using the unique "primer tRNA" binding specificity. (4) Characterization of the specific poly(A)RNAs, including messenger RNA activity by protein synthesis assay and possible changes of level during neoplastic process.

Major Findings: A method for demonstrating primer tRNA binding poly(A)RNA molecules has been established. The experimental procedures include poly(A)RNA isolation, agarose gel electrophoresis, RNA transfer to DBM-paper and molecular hybridization. With this method, it is now possible to demonstrate various specific poly(A)RNA molecules in cultured human cells. Initial studies indicated that a human breast cancer cell line (ALAB) contained a unique primer tRNA binding RNA molecule of 24S size. This molecule is present in all human breast cancer epithelial cell lines examined, but not in epithelial cell lines of other

human cancers, nor in the fibroblast cells derived from human breast cancers. It apparently binds selective tRNA species, the identity of which is being investigated. In the reverse transcription reaction, a copy DNA of about 140 base lengths linked to a 70 base RNA primer can be demonstrated. Preliminary results suggest that content of this RNA molecule in the ALAB cells may be stimulated by hydrocortisone treatment.

Work on the molecular binding using plasmids and the Charon series of λ phage and of in vitro translation using the rabbit reticulocyte system have been initiated to characterize the 24S RNA molecule of human breast cancer cells. Employing a molecular clone of mouse rRNA gene, the sites of proline tRNA binding at the 3' portion of mouse 28S rRNA have been located. This shows that selective tRNA·rRNA interaction (in an in vitro hybridization reaction), is not due to nonspecific binding. Because of the vast sequence heterogeneity and possible marked evolutional changes of the LTR (large terminal repeats) of retroviral DNA, it is apparent that the primer tRNA approach may be useful for the detection and isolation of this class of transposable gene elements in human cells. Also, the focus of this study has shifted from human cancer cells in general to some specific tumor cell types, particularly from cancer, which show positive results in the search for specific retroviral RNA expression.

Significance to Biomedical Research and the Program of the Institute:
One of the objectives of the Program is detection of tumor viral information in human cancer. To date, partial expression of retrovirus-like information, but rarely complete virus, has been detected in human leukemias, breast carcinomas, and melanomas, as well as normal fibroblasts and placental tissues; however, only partial homology to retroviruses of other species has been described. This project offers a new approach to the detection of partial viral information, as well as to study restriction mechanisms operative in human cells.

Proposed Course: Experiments will continue that utilize tRNAs as ligands in affinity chromatography for separation of cellular poly(A)RNAs. Further studies concerning the detection and isolation of retroviral transposable gene elements in human cells will be performed.

Date Contract Initiated: February 1, 1979

Current Annual Funding: $182,160

HARVARD COLLEGE (NO1-CP8-1004)

Title: Biological Studies of FOCMA Expression: Biochemical Characterization of FOCMA

Contractor's Project Director: Dr. Myron Essex

Project Officer (NCI): Dr. Takis S. Papas

Objectives: (1) Conduct biological experiments to characterize feline oncornavirus cell membrane antigen (FOCMA) as a product of the feline sarcoma virus (FeSV) src gene. (2) Conduct biochemical studies to characterize FOCMA as a product of the FeSV src gene.

Major Findings: Spontaneous and FeSV-induced tumor cells were examined after explantation in vitro for expression of the FeSV-related "gag-x", p110, polyproteins. Those transformation-specific proteins related to each strain of FeSV were regularly found in cells from tumors induced by that strain, but no "gag-x" proteins were found in spontaneous tumors that were unrelated to FeLV-FeSV. The proteins were expressed in cat cells that produced FeLV as well as in non-feline nonproducer cells (mink, rat) as described earlier. It was determined that cats could respond to the "X" portion of "gag-x" and that such "X" specific sera give a typical FOCMA type reaction on lymphoid cells. Murine and cat cells were transfected with restricted and unrestricted FeSV proviral DNA and transformed with the induction of ST-FeSV "gag-x". Some transfection studies were done with DNA from feline lymphomas and an unusual "strain" of FeLV was isolated based on host-range criteria. FeSV was used to induce nonproducer tumors in rats and such tumor cells were found to contain "gag-x". Using the fluorescence activated cell sorter, it was demonstrated that all of the FeLV virion structural proteins were expressed at the cytoplasmic membrane of cultured producer lymphoma cells. Several candidate monoclonal antibodies were examined for activity to "gag-x". Nonviremic cats that were naturally exposed to FeLV and had high FOCMA antibody titers were found to maintain those high antibody titers in the absence of re-infection, suggesting the possibility that FOCMA positive cells may persist in some virus-negative animals.

Significance to Biomedical Research and the Program of the Institute: Purification and characterization of oncornavirus virion-associated antigens has proceeded to a high degree of sophistication for murine, avian and feline oncornaviruses. However, the product of the src gene, presumably the "transforming protein" which is thought to be a key substance required for malignant transformation of vertebrate cells by viruses, has not been identified biochemically or immunologically. FOCMA antigen is perhaps the best described tumor-specific cell surface antigen which occurs in any oncornavirus system. It has the additional distinction of being an antigen which is involved in a major way in dictating the outcome of naturally occurring diseases in an outbred animal population. The information obtained in such work may be applicable to prophylaxis against other animal oncornaviruses and may possibly have particular future value in control of some human neoplastic diseases.

Proposed Course: This contract terminated September 28, 1981.

Date Contract Initiated: September 29, 1978

Current Annual Funding: No funding in FY 81

JACKSON LABORATORY (NO1-CP3-3255)

Title: Natural Occurrence of RNA Tumor Viruses (Genomes) and Host-Gene Control of their Expression

Contractor's Project Director: Dr. Hans Meier

Project Officer (NCI): Dr. Stephen J. O'Brien

Objectives: The primary objective of this contract is to achieve an understanding of the mechanisms underlying the genetic determination of susceptibility and

resistance to cancer and the RNA tumor viruses. The Jackson Laboratory is a unique source of highly inbred mouse strains. These are used to define specific gene influences on type C RNA virus/genome/tumor expressions under natural conditions, and the influence of environmental and other factors (carcinogens, aging) on host gene controls of oncogene and virus expressions.

Major Findings: BXH-2 mice develop a non-thymic leukemia around seven months of age. Strain BXH-2 carries the Fv-1 allele, and spontaneously releases a B-tropic MuLV throughout its life; xenotropic MuLV is first detectable at 2 months of age and its titer increases with age. Leukemic tissues contain several types of dualtropic MuLV; one such isolate is B-tropic and induces cytopathic changes in mink lung and mouse fibroblast cells. Its role, if any, in BXH-2 leukemia is as yet unknown.

Linkage has been established between the Lyb-4 alloantigen locus and the chromosome 4 markers Lyb-2 and Mup-1 using RI strains of mice. While the antigenic determinant on DBA/2J mice is indistinguishable from that of C3H/HeJ mice, the C3H/HeJ gene appears to be nonallelic and unlinked to the DBA/2J gene.

ENU causes both teratogenic and carcinogenic effect in rabbits when administered transplacentally. Since evidence for a 70S RNA associated with RDDP activity in uterine tissues had previously been identified, the expression of replication-defective endogenous type C viral RNA may serve as a useful marker for the effects of chemical carcinogens.

Studies have continued to determine the cause(s) of the divergence in leukemia incidence between hairless (hr/hr) and haired (hr/+) mice of the HRS/J strain. Previous observations have implicated genotype-dependent differences in the immune system and allelic disparities in endogenous MuLV titers. Using the transplantable lymphoid tumor HTR, it was found that hr/+ mice survive significantly longer after inoculation than do hr/hr mice, suggesting a greater ability of hr/+ mice to mount an immune response against tumor antigens. Also, immunization of hr/+ mice resulted in a signficantly prolonged life relative to unimmunized hr/+ mice, whereas hr/hr mice did not develop a protective immune response to the tumor.

The T suppressor cell alloantigen Ts^d has been found to map near the immunoglobulin allotype genes and may be an allotype marker on T cell receptor immunoglobulin.

Significance to Biomedical Research and the Program of the Institute:
It is now possible, genetically, to ameliorate cancer in a mouse by breeding. By identifying the genes and loci involved in cancer susceptibility and the immunological and physical markers associated with the "high cancer" genes, it is now possible not only to identify the highly susceptible animal, but to study the biochemical, immunological and metabolic mechanisms controlled by these genes. The development of this information will help (1) elucidate markers to identify the cancer-prone individual, and (2) determine how genes operate in regulating the natural oncogene, with the goal of correcting deficiencies associated with the switching on of cancer cells in susceptibles.

Proposed Course: This contract terminated May 31, 1981.

Date Contract Initiated: May 2, 1967

Current Annual Funding: No funding in FY 81

JOHNS HOPKINS UNIVERSITY (NO1-CP8-1053)

Title: Studies on the Molecular Biology of Oncornaviral Proteins

Contractor's Project Director: Dr. J. Thomas August

Project Officer (NCI): Dr. John R. Stephenson

Objectives: To purify and characterize proteins specific to oncovirus transformed cells, purify and characterize virion proteins of murine endogenous xenotropic oncoviruses, and study endogenous viral expression and recombinant viruses.

Major Findings: A 55,000 dalton rat cell membrane glycoprotein, gp55, has been purified to homogeneity and characterized. This protein was originally identified in preparations of a defective pseudotype of the Kirsten sarcoma virus and shown to be present in several rodent retrovirus particles. The gp55 was purified from this defective virus by conconavalin A and heparin affinity chromatography as well as by preparative SDS-gel electrophoresis. The ^{125}I-labeled gp55 was precipitated by antisera against rodent retroviruses, but not by monospecific antisera against purified type C virus structural proteins, thus indicating that gp55 was retrovirus associated, but unrelated to known retrovirus structural proteins. Competition radioimmunoassay indicated: (1) the presence of gp55 antigens in 15 rodent cell lines, but not 10 non-rodent cell lines; (2) no effect of viral infection or cell transformation on the amount of gp55 expressed; (3) up to 100 fold increases in the concentration of the gp55 antigens in 9 rodent retroviruses, but not in 5 non-rodent viruses, as compared to cells; (4) the presence of gp55 in rodent sera, especially of the NZB mouse, where anti-gp55 antibody was also detected; and (5) a lymphoid and epithelial tissue distribution of gp55 in rats and mice. In conclusion, gp55 is a cell membrane glycoprotein associated in high concentration with retroviruses.

A 20,000 dalton, transformation-related, rat cell membrane protein has been purified to homogeneity. This protein, p20, was originally identified in preparations of a defective woolly monkey leukemia virus pseudotype of Kirsten sarcoma virus. The chromatographically purified p20 was an acidic, hydrophobic protein, capable of specifically binding GTP (K_D = 15 μM). This nucleotide binding property and other previously reported characteristics were similar to properties ascribed to the Harvey sarcoma virus src gene product. The p20 protein also appeared similar to this src gene product when immunoprecipitates of both proteins were directly compared by one- and two-dimensional $NaDodSO_4$ gel electrophoreses. However, the proteins were not identical since their tryptic maps differed. Using a competition radioimmunoassay, the concentration of p20 in cells, viruses, and rat tissues has been measured. The p20 protein was not encoded by rat sarcoma viruses because it was increased only slightly following Kirsten sarcoma virus transformation of rat cells and was not increased in non-rat cells transformed by the Kirsten or Harvey sarcoma virus. Remarkably, of ten rat tissues examined, p20 was found predominantly in brain, specifically in the membranes.

Significance to Biomedical Research and the Program of the Institute:
One of the principal areas of interest in viral oncology is the identity and function of viral-coded transforming proteins. Procedures have been developed that enable the purification under nondenaturing conditions of all major structural

proteins of Rauscher and Gross murine oncornaviruses; monospecific antisera have been prepared in goats against each, thus providing valuable reagents for studies in the structure, function and expression of viral proteins. Preparation of similar reagents for the xenotropic murine viruses and primate viruses is important for extension of this approach to these important viruses.

Proposed Course: This contract terminated August 31, 1981.

Date Contract Initiated: September 1, 1978

Current Annual Funding: No funding in FY 81

MICHIGAN CANCER FOUNDATION (NO1-CP8-1001)

Title: Effects of Environmental Factors on Expression of Endogenous MuMTV in Low Mammary Strains of Mice

Contractor's Project Director: Dr. Charles M. McGrath

Project Officer (NCI): Dr. Robert Callahan

Objectives: To determine whether exogenous mammotropic hormones can change MuMTV expression in mammary cells and whether the change correlates with tumor onset and incidence in low mammary strains of mice.

Major Findings: In vitro translation of RNAs from D2 hyperplastic alveolar nodules (HAN), D2 tumors and BALB/cfC3H tumors resulted in the synthesis of p28, while BALB/cfC3H tumor mRNA translated into similar amounts of the core and envelope polypeptides. The 25S RNA fraction from the BALB/cfC3H tumors directed the synthesis of gp52, while 35S and 13S did not. Synthesis of gp52 was not detected in the translation products of 25S RNA from either D2 HAN or D2 tumors. It was concluded that either endogenous MuMTV in BALB/cfC3H D2 tissues expresses a defective envelope mRNA, specific factors are required for its translation, or its translation product is antigenically unrelated to MuMTV-S envelope and escapes immunodetection. An antigenically altered endogenous MuMTV envelope gene product, by analogy to certain murine type C recombinant transforming viruses, could have important implications regarding the oncogenic potential of endogenous MuMTV in mammary epithelium.

Significance to Biomedical Research and the Program of the Institute: It may be possible to regulate human breast tumor incidence by controlling the hormonal environment of the host. This study is designed to elucidate the role of viral-hormonal interactions in the generation of animal mammary tumors and attempts will be made to manipulate both factors in vivo.

Proposed Course: This contract terminated January 31, 1981.

Date Contract Initiated: February 1, 1978

Current Annual Level: No funding in FY 81

MOUNT SINAI SCHOOL OF MEDICINE AND HOSPITAL (NO1-CP4-3225)

Title: Stimulation of Immunity to Virus-Associated and Tumor-Associated Antigens in Mouse Systems

Contractor's Project Director: Dr. J. George Bekesi

Project Officer (NCI): Dr. Paul H. Levine

Objectives: (1) To evaluate chemo-immunotherapeutic measures in tumor-bearing animals using viral inhibitors to determine the best means for controlling the oncogenic virus and the tumor. (2) To determine the presence of viral antigen and/or specific antivirus immunity in experimental animals and untreated controls. (3) To transfer the knowledge gained from these animal studies to clinical applications against human cancer.

Major Findings: Assessment of the interrelationship of mammary cells and the replication of mouse mammary tumor virus (MuMTV) was initiated by morphological and biochemical analyses of the effects induced by cytochalasin B (CB) or colcemid. CB and colcemid did not affect the morphology of budding or extracellular particles although CB reversibly interrupted microvilli formation. CB reversibly decreased MuMTV production by altering the kinetics of virus replication; MuMTV-associated protein increased 2-fold following CB removal. SDS-PAGE polypeptide profiles of MuMTV revealed alterations in the relative composition of the virions. Colcemid treatment increased MuMTV production, altered the relative levels of MuMTV polypeptides, and resulted in the incorporation of an apparently heavily glycosylated form of gp60. These results indicated that both microtubles and microfilaments participate in MuMTV replication. In addition, the 44,000 dalton protein (p44) in MuMTV was shown to have the same electrophoretic mobility as actin and also possessed ATP and ion-dependent polymerization activities of actin. Incorporation of actin into the virus was specific and the data suggested that p44 may be structurally associated with the MuMTV glycoprotein, gp37.

Significance to Biomedical Research and the Program of the Institute: Evidence suggests that in acute leukemia, breast cancer, and Burkitt's lymphoma, late relapse may actually be disease reinduction due to persistence of the factors initially associated with the disease. If there is a viral etiology for these three diseases, it is important to obtain methods of permanently controlling the virus while chemotherapy or immunotherapy are used to control the tumor itself. Successful development of treatment protocols in animals with leukemia, breast cancer, and lymphoma are an important first step in curing these forms of human cancer.

Proposed Course: This contract terminated December 31, 1980.

Date Contract Initiated: August 6, 1973

Current Annual Level: No funding in FY 81

NETHERLANDS CANCER INSTITUTE (NO1-CP3-3368)

Title: Immunogenetic Studies on Breast Cancer and Leukemia

Contractor's Project Directors: Dr. J. Hilgers

Project Officer (NCI): Dr. Ernest J. Plata

Objectives: To study the immunogenetics of mouse mammary tumor and MuMTV transmission, as well as that of leukemia and murine genes for transmission, MuMTV replication, histocompatibility, and immune response.

Major Findings: Purified MuMTV preparations from milk and mammary tumors of high cancer strains, such as the C3H, DBA, RIII, WLL and also the low incidence strain BALB/c infected with its own endogenous virus, were titrated in many inbred strains and their host range determined. It appears that they can all be distinguished by this criterion and that they all may be slightly different from each other. The influence of the H-2 locus on exogenous MuMTV induced mammary cancer in mice was extensively studied.

Somatic cell hybrids were generated between E36 Chinese hamster lung fibroblasts and GRSL cells (EGR hybrids) as well as primary GR mammary tumor cells (EMT hybrids). Both series segregate mouse chromosomes and can be used to map mouse genes, such as the germinal and somatic (amplified) proviruses. These hybrids do not support expression of MuMTV, although the mouse parental cell is known to produce high amounts of MuMTV proteins (either as A particles in case of GRSL or as A and B particles in case of GR mammary tumor cells). Apparently expression of MuMTV depends on the "differentiation" state of cells. Dexamethasone is able to induce MuMTV in the EMT hybrids and the inducible locus is being mapped.

Significance to Biomedical Research and the Program of the Institute:
Study of genetic factors controlling host susceptibility and capacity for internal control of oncogenic virus expression and infectivity is important to the program of this Institute. The results obtained thus far begin to delineate a variety of factors, in addition to the presence of an oncogenic virus, which contribute to or modify the pathogenesis of mammary cancer. The findings described contribute to the investigations of possible etiological agents of human cancer as well as to greater understanding of the fundamental biology of cancer.

Proposed Course: This contract terminated February 28, 1981.

Date Contract Initiated: June 28, 1972

Current Annual Level: No funding in FY 81

OHIO STATE UNIVERSITY RESEARCH FOUNDATION (NO1-CP9-1008)

Title: Immunoprevention of Cancer in Cats

Contractor's Project Director: Dr. Richard G. Olsen

Project Officer (NCI): Dr. Garrett V. Keefer

Objectives: To determine: (1) the efficacy of feline oncornavirus-associated cell membrane antigen (FOCMA) vaccine for protection of SPF kittens against feline leukemia virus (FeLV) -induced disease, (2) the immunosuppressive properties of FeLV virion polypeptides, (3) the autogenous immunity of cats to

various FeLV virion antigens and FOCMA and conduct immunologic surveillance of humans exposed to feline oncornaviruses.

Major Findings: The efficacy of feline leukemia soluble tumor cell antigen vaccine (STAV) has been confirmed in conventional cats (non-SPF). This vaccine was tested in a large multi-cat household. All vaccinates produced FOCMA antibody and the degree of immunoprotection was equivalent to that reported early with SPF cats ($\gtrsim$ 80%).

An assay was developed to measure suppressor cell function in cats. Studies to date indicate the cats infected with FeLV exhibit striking loss of suppressor cells. Loss of suppressor cell function in cats infected with FeLV corresponds with other lymphocyte functions such as blastogenesis to lectins. Recently, the contractor developed a one-way mixed lymphocyte reaction (MLR) test for cats.

The immunosuppressive retroviral protein (FeLV p15E) abrogates the MLR reaction. Retroviral protein from RD-114 virus (RD-114 p20) also interferes with lymphocyte functions including lymphocyte capping, lymphocyte blast transformation and MLR.

Work is in progress to delineate the mode of immunosuppressive action of FeLV p15E. Presently, the contractor is focusing on FeLV p15E binding T lymphocytes, integration into cell membranes and effects on cyclic nucleotide levels in normal cells. Moreover, work is in progress for the production of monoclonal FeLV p15E. This reagent will aid in delineating the effect of FeLV p15E in cellular functions. In another aspect of the p15E studies, a recombinant FeLV/RD114 virus is being evaluated for pathogenic and immunosuppressive properties. The recombination site appears to be within the env gene of FeLV and may involve the p15E region. To date, 5 of 5 newborn kittens challenged with the recombinant virus have developed chronic viremia and 4 of 5 have died.

Significance to Biomedical Research and the Program of the Institute: This project addresses immunoprevention of cancer in an important animal model, the cat. Cats share man's environment, and it is very likely that the same cocarcinogenic principals would prevail in both cat and human cancer. In the feline system, both host and virus components can be well controlled. Results pertaining to cause and prevention of cancer in a laboratory setting utilizing an outbred animal system can be transferred with established techniques to natural animal and human populations.

Proposed Course: This contract terminated September 30, 1981.

Date Contract Initiated: October 1, 1978

Current Annual Funding: $174,250

SCRIPPS CLINIC AND RESEARCH FOUNDATION (NO1-CP9-1012)

Title: Genetic Analysis of Immune Response of Mice to Recombinant gp70 Oncornavirus

Contractor's Project Director: Dr. Richard A. Lerner

Project Officer (NCI): Dr. Garrett V. Keefer

Objectives: Correlate genetic control of immune responsiveness of inbred mouse strains to different murine oncornavirus gp70 determinants; determine relative contributions of humoral immunity and cell-mediated immunity in affording protection against tumor antigens and tumor growth; induce tumors in appropriate parental strains with cloned characterized N- and B-tropic oncornaviruses, then test the tumors for growth in F_1 hybrids; identify distinct members of the gp70 family to which Ir gene controlled responses are directed; test F_1 hybrid mouse strains rejecting parental N-tropic virus-induced tumor for ability to reject parental tumor induced by an N-tropic recombinant virus; and determine the molecular basis for immune responses directed to molecules other than gp70.

Major Findings: Studies have identified two types of cell mediated cytolytic activity exhibited by AKR spleen cells obtained from animals which have been previously primed with lethally irradiated tumor cells. The first does not require in vitro stimulation with tumor cells and is also found in normal AKR mice. It appears refractory to the effects of anti-theta and complement, antigen-antibody complexes, and is not affected by the removal of adherent cells. These results imply such reactivity is mediated by natural killers. The second type of activity requires in vitro challenge with irradiated tumor cells.

In order to address the range of specificity of these killer lymphocytes, a panel of different AKR thymomas has been used. It was found that, irrespective of the syngeneic thymoma used for stimulation, there appeared to be a hierarchy of target susceptibility to lysis by the responding cells. Thus, stimulating with SL7, SL8 or BW5147 tumors (all spontaneous AKR thymomas) resulted in a greater percentage lysis of BS5147 than SL7, SL7 than SL8, and SL8 than SL2 or SL3. Studies concerning the poor or total lack of responsiveness to the AKR thymomas SL2 and SL3, found that if one uses the panel of AKR thymomas as allogeneic stimulators, it was discovered that SL2 and SL3 (the poorest syngeneic targets) do not induce an allogeneic cytotoxic response (Table 1). This result is inferential of there being abnormal expression of histocompatibility antigens on the SL2 and SL3 cell lines. A further abnormality of these two cell lines is that the major expression of the gp70 glycoprotein is of the ecotropic type murine leukemia virus as opposed to the recombinant type gp70 expressed in BW5147, SL7 and SL8 cell lines. Thus, there is an apparent association between abnormal histocompatibility antigen expression and endogenous virus expression.

Significance to Biomedical Research and the Program of the Institute: One of the principal areas of interest in viral oncology is the identity and function of the viral coded transforming proteins. Detailed structural analysis of murine oncornavirus virion proteins has enabled production of highly specific immunological probes for these proteins. The functional role these proteins play in virogenesis and oncogenesis and cellular control of the synthesis and functioning of these proteins can now be probed as model systems.

Proposed Course: Experiments will continue to determine how the murine system copes with a viral antigenic repertoire which is changing and needs to know about both antibody and cellular mediated killing. Studies are currently underway to determine the effector cell type involved.

Date Contract Initiated: November 1, 1978

Current Annual Funding: $70,840

SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH (NO1-CP7-1059)

Title: Structure and Distribution of Integrated Sequences of Feline Oncornaviruses

Contractor's Project Director: Dr. Wolf Prensky

Project Officer (NCI): Dr. Stephen J. O'Brien

Objectives: To determine the integration sites of feline leukemia virus (FeLV) and endogenous virus (RD-114) in cat cells by somatic cell hybridization and in situ hybridization techniques.

Major Findings: Twenty monoclonal antibodies to FL74 cell FOCMA were used to probe its expression in: (a) lymphosarcoma (LSA) cells, (b) FeSV-transformed fibrosarcomas, (c) non-transformed cat fibroblasts injected with different subgroups of FeLV, (d) cells infected with FeLV from several LSA cells, and (d) cat-rodent somatic hybrid cells. Differential reactivity of the antibodies established the existence of four main groups of epitopes, the largest group being common to LSA fibrosarcomas, and FeLV-C infected fibroblasts. The data showed that (1) FeLV-C codes for a large number of FOCMA epitopes, (2) there are some FOCMA epitopes (Group II) not encoded by FeLV-C, and (3) LSA cells with and without FeLV-C contain a nucleic acid which is generally not transmitted by viral infections. Findings also imply that most cat antibodies to FOCMA also neutralize FeLV-C, and that in FeLV-A infected cats similar recombination events may be responsible for generating RNA molecules encoding FOCMA and FeLV-C.

The segregation of FeLV due to loss of cat chromosomes could be observed in hybrids from 5 LSA cell lines. All hybrids expressed some normal cat antigens. FOCMA expression, as monitored by antibody 1-539, was readily detected in only two out of 10 segregating populations. The low frequency of FOCMA expression could only partially be attributed to the presence of a single coding site in the genome.

Mouse 3T3-4C2 and Chinese hamster GRC^{+}-LR73 cells were fused with 3281 LSA cells to test for the expression of the transformed phenotype in somatic cell hybrids. No anchorage independent hybrids were obtained from cat-mouse but large numbers were obtained from cat-hamster fusion experiments. The GRC^{+} cells appeared to permit expression of the transformed phenotype of leukemic cells. Cells from a number of anchorage independent foci were tested for FeLV and FOCMA expression, both of which were detected. At least one clone was negative for FeLV and expressed FOCMA determinants.

Because 3403 cells expressed Group IV antigens, they were checked to determine if they contained FeLV-C. Eight weeks of repeated infection were needed to demonstrate FeLV-C. The occurrence of FeLV-C is probably more frequent than indicated by previous studies. In cat LSA cells, it is masked by low titer, and in many cats by neutralizing antibodies.

Significance to Biomedical Research and the Program of the Institute:
It is possible that the site of FeLV integration may be responsible for the consequences of infection. Interaction with endogenous RD-114-related genetic information may also play a role. This project will use the various types of tumors of the cat as input material for studying the integration sites of FeLV and RD-114 at the chromosomal level to determine whether the nature of the

integration site(s) play any role in oncogenicity or expression of the viral genome.

Proposed Course: This contract terminated March 27, 1981.

Date Contract Initiated: September 28, 1977

Current Annual Funding: No funding in FY 81

SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH (NO1-CP8-1054)

Title: Immunogenetic and Virological Study of Leukemogenesis in the AKR Mouse

Contractor's Project Director: Dr. Lloyd J. Old

Project Officer (NCI): Dr. Stephen J. O'Brien

Objectives: (1) Extend present serological typing systems to identify new cell surface antigen systems specified by various classes of endogenous MuLV. (2) Characterize new antigens biochemically and determine relationship to viral structural components. (3) Conduct detailed immunogenetic and virological analyses of preleukemic phase of AKR mice. (4) Use genetic approach to analyze age-dependent amplification of MuLV antigenic expression in thymus of six month old AKR mice. (5) Determine biological and biochemical character and significance of MuLV isolated from AKR mice in (3) above. (6) Use cell fractionation techniques to identify the cellular source of various classes of MuLV from thymus of different age AKR mice.

Major Findings: A monoclonal antibody of a cell surface antigen of the mouse related to the xenotropic class of MuLV ($G_{(ERLD)}$) has now been characterized and its reaction parallels precisely the results obtained with conventional antibody. A new series of antisera produced in RF mice against AKR leukemia cells are under analysis and 2 individually distinct antigens restricted to the immunizing leukemia cells have been identified. Since this category of cell surface antigens has not been found on leukemia cells the elucidation of the biochemical nature and genetic origin of these antigens is being performed.

Rat monoclonal antibodies to gp70 determinants of ecotropic MuLV have defined 7 epitopes on the molecule and these reagents are proving useful in characterizing different dualtropic MuLV. One of the monoclonal antibodies detects an epitope resembling the G_{IX} determinant, and this antibody reacts with a region of the gp70 molecule apparently involved in viral attachment to the cell surface as indicated by its viral neutralizing activity. With regard to the characterization of MuLV from young preleukemic and leukemic AKR mice, the following points were established: (a) dualtropic MuLV, but not ecotropic or xenotropic MuLV, are capable of amplifying expression of MuLV-related antigens on thymocytes (A^+ trait) and of accelerating leukemia development (L^+ trait); (b) both A^+L^- and A^-L^+ dualtropic MuLV isolates were identifed, indicating that these 2 activities of antigen amplification and leukemia acceleration represent separable viral phenotype; (c) dual tropic viruses can be classified serologically into 3 distinct phenotypes; however, no serological markers have been found to distinguish A^+L^- from A^-L^+ MuLV; (d) a single infectious unit of A^-L^+ MuLV (measured by in vitro assay) was capable of antigen amplification in vivo; and (e) analysis of the

surface antigenic phenotype of amplified thymocytes or accelerated leukemias indicates direct coding by the input virus rather than by new MuLV species resulting from additional recombinational events after infection and leukemic transformation.

A surprising outcome of a survey of MuLV for leukemia accelerating activity in AKR mice was the finding that a nonleukemogenic dualtropic MuLV isolated from Moloney MuLV, designated SMX-1, actually inhibited the development of spontaneous (as well as MuLV-accelerated) leukemia. Intrathymic injection of SMX-1 resulted in marked reduction in the incidence of spontaneous leukemia; 35% at 1 year as compared to an incidence of 98% in control AKR mice. SMX-1 had no inhibitory activity when injected by the IP route but was effective after IV injection. Protection appeared maximal when SMX-1 was injected into 1- or 2-month-old mice. Less protection was seen when mice were injected at 110 days and no protection if injection was delayed until 6 months of age. An important question for future investigation is whether SMX-1-mediated inhibition of leukemia is restricted to AKR mice or whether leukemia development induced by x-ray or chemical carcinogens in other strains of mice might also be prevented.

Significance to Biomedical Research and the Program of the Institute: Detailed structural analysis of murine oncornavirus virion proteins has enabled production of highly specific immunological probes for these proteins. The functional role these proteins play in virogenesis and oncogenesis and cellular control of the synthesis and functioning of these proteins can now be probed.

Proposed Course: This contract terminated September 24, 1981.

Date Contract Initiated: September 25, 1978

Current Annual Funding: No funding in FY 81

SOUTHERN CALIFORNIA, UNIVERSITY OF (NO1-CP8-1032)

Title: Immunoprevention of Natural and Induced Tumors in Wild Mice

Contractor's Project Director: Dr. Murray B. Gardner

Project Officer (NCI): Dr. Stephen J. O'Brien

Objectives: By infection with helper amphotropic MuLV, attempt to rescue the transforming genes from chemically transformed cells and chemically induced tumors of wild mice and New World rodents; determine the role of amphotropic virogenes in natural and chemical tumorigenesis of laboratory and wild mice; attempt to prevent chemically induced tumors by passive immunization with anti-amphotropic IgG; determine interaction of amphotropic and ecotropic murine leukemia viruses (MuLV) with lymphoid cells during the pathogenesis of natural and experimental B cell lymphomas of wild mice; study the role of ecotropic MuLV virogenes in slow CNS disease in wild mice and attempt passive immunization with anti-ecotropic IgG; determine the role of recombination of amphotropic and ecotropic MuLV in the genesis of viral isolates with enhanced oncogenicity; attempt to determine the mechanism of control of amphotropic virus expression in tissues of laboratory mice.

Major Findings: The Akvr-1^R gene that is polymorphic in wild mice restricts in vivo replication of ecotropic MuLV from the following inbred mice: AKR, C58, PL/J, C3H/Fg (all N-tropic), B10-Y (B-tropic) and Friend and Moloney MuLV (NB-tropic). This universal effect suggests that the ecotropic viruses from American laboratory mice are closely related in their restriction target. Akvr-1 also restricts wild mouse ecotropic virus in vivo and, thus, segregation of this gene may determine risk to paralysis and lymphoma in individual Lake Casitas (LC) wild mice. The Akvr-1^R gene does not restrict amphotropic MuLV of LC mice (N-tropic), xenotropic MuLV of NZB mice or mammary tumor virus of C3H/He mice; nor does this gene inhibit chemical tumorigenesis. The in vitro restriction upon exogenous virus infection is exerted more strongly in hematopoietic than fibroblastic cells. In fibroblastic cells the restriction occurs at or before the stage of reverse transcription. Whether or not this gene represses transcription of endogenous AKR virogenes is, as yet, undetermined. By crossing LC (Akvr-1^{RR}) mice with AKR mice congenic for Fv-1^b and backcrossing the progeny to AKR (Fv-1^n), it has been proven that the Akvr-1 and Fv-1 loci are different and non-linked. Akvr-1 has not yet been mapped, but the locus is not located close to certain retrovirus structural (Xv-1, Akv-1, Fvg-1) or regulatory (Rec 2, Ram 1, Fv-1, Fv-2) loci. The Akvr-1 and Fv-4 loci appear to be closely related and possibly identical.

Genomic characterization of wild mouse amphotropic and ecotropic viruses by RNase T_1 oligonucleotide mapping showed extensive homology although sequence differences were detected in the env gene region. A greater sequence divergence was observed between ecotropic than between amphotropic isolates.

Naturally occurring lymphoma of LC wild mice and the lymphomas induced by LC MuLV (amphotropic or ecotropic) in NIH Swiss mice were composed of null cells lacking T- and B-cell markers. Two LC lymphomas that were established in vitro retained surface and cytoplasmic IgA, and yet another culture consisted mainly of macrophages. As a model for humans, the LC lymphomas most closely resemble childhood acute lymphoblastic leukemia, which often consists of a leukemic proliferation of null cells.

A laboratory colony of LC wild mice which remain consistently free of endogenous MuMTV virogenes has been derived. These mice show no impairment of breeding or lactation.

Significance to Biomedical Research and the Program of the Institute: Development of methodology for derepressing the genes governing expression of the complete retrovirus from laboratory mouse strains could be applied to the rescue of a human virus. Further, the lymphomas associated with the wild mouse type C virus are of a B cell type, whereas lymphomas in laboratory mouse models, T cell lymphomas are more common. Human lymphomas are also of B cell type; therefore, the outbred wild mouse model has great relevance for study of the human disease.

Proposed Course: This contract terminated September 15, 1981.

Date Contract Initiated: September 16, 1978

Current Annual Funding: No funding in FY 81

SOUTHERN CALIFORNIA, UNIVERSITY OF (NO1-CP8-1033)

Title: Rescue of Human src Genes and Identification of Related Tumor Antigens

Contractor's Project Director: Dr. Murray B. Gardner

Project Officer (NCI): Dr. Steven R. Tronick

Objectives: To identify families with history of colon polyposis, Gardner's syndrome, and other genetically related cancers; obtain tissues from the tumor patients and/or families; establish cultures and characterize growth properties. Compare fibroblast cell cultures for transformability by KiMSV, rat sarcoma virus BaEV pseudotype, RD-114 pseudotype and amphotropic murine pseudotype. Attempt to rescue human and primate transforming (src) genes from spontaneous tumors and from human and primate cells transformed by chemicals and/or DNA viruses.

Major Findings: Growth characteristics and susceptibility to viral transformation were compared in cultured skin fibroblasts from patients with Gardner's syndrome (GS), those with familial polyposis coli (FP), asymptomatic family members, and unrelated controls. Compared to cells from unrelated controls, cells from 4 of 5 GS patients were tranformed by Kirsten murine sarcoma virus at 100- to 1000-fold increased efficiency. The transformation efficiencies of fibroblasts from 2 of 3 FP patients were 10- to 100-fold greater than those of unrelated controls. However, because the fibroblasts from some of the young asymptomatic GS and FP family members also transformed at higher efficiency than did cells from unrelated controls, long-term observation of these families is required. This would determine whether or not these members develop clinical manifestations of GS or FP and thus establish the specificity of this assay for detection of individuals bearing the mutant gene. Compared to fibroblasts from unrelated controls, GS and FP fibroblasts showed a two-fold to three-fold increased saturation density and plating efficiency, but this difference was not noted with fibroblasts from most of the asymptomatic family members.

The plating efficiency of GS and FP fibroblasts was not enhanced by treatment with the tumor promoter TPA. Nor were the treated cells morphologically altered. Therefore, this TPA assay cannot be used to distinguish these individuals from controls.

The oligonucleotide pattern of 60-70S viral RNA from RD-114 and BaEV showed that, although these viruses are genetically distinct, they do share some sequence homology, i.e., 10 or 45 large oligonucleotides. Most of this genomic similarity appears in the 5' terminal region.

Significance to Biomedical Research and to the Program of the Institute: Isolation and characterization of a primate transforming gene is the first step toward developing rational immunological control of human cancer. Additionally, as more experience is gained, the KiMSV transformation assay may be useful in identifying individuals at risk in dominant cancer gene families.

Proposed Course: This contract terminated September 15, 1981.

Date Contract Initiated: September 16, 1978

Current Annual Funding: No funding in FY 81

SOUTHERN CALIFORNIA, UNIVERSITY OF (NO1-CP8-1063)

Title: Immunoprevention of Cancer in Cats

Contractor's Project Director: Dr. Murray B. Gardner

Project Officer (NCI): Dr. Garrett V. Keefer

Objectives: To determine the interrelationship of RD-114, FeLV and src gene expression to FOCMA antigen in naturally occurring and chemically induced feline cancer; to determine if transformation-specific src genes can be rescued as sarcoma viruses from cat tumors; to determine the relationship between FOCMA and the hypothetical feline src gene transforming protein; to determine the correlation between autogenous immunity to FeLV or FOCMA and occurrence of disease; to determine whether cats can be protected against natural or induced cancer by immunization with FOCMA and/or feline transforming protein; and to determine whether humans show any evidence of infection after exposure to FeLV.

Major Findings: Feline sarcoma virus (FeSV) has been isolated from another spontaneous fibrosarcoma of a domestic cat. This new independent FeSV isolate has been transmitted serially in vivo and in vitro. It induced fibrosarcomas in fetal and young kittens with a latent period of about two months and induced foci of transformed fibroblasts (cat, dog, mink) in vitro after 5-7 days. Filtered supernatants also induced sarcomas in kittens. Several nonproductive clones of FeSV-transformed goat cells have been derived. The ratio of helper to sarcoma virus, the FeLV subgroup, and the relationship to src genes of the other three extant FeSV strains will soon be determined.

FeSV src antibody was not detected by immunoprecipitation in any natural FeLV-immune sera, including many that were strongly positive for FOCMA antibody. Nor was src antibody found in any of 14 cats with spontaneous sarcomas, including two cats from whose sarcomas FeSV was isolated. By contrast, src antibody was readily detected by the same technique in cats which were experimentally inoculated with Gardner-Arnstein (GA)-FeSV. It was concluded that humoral immunity to feline src protein does not account for protection of cats against the natural occurrence of tumors caused by either FeLV or FeSV.

The ELISA test correlated closely with the FA test for diagnosis of FeLV infection. However, the ELISA procedure appears slightly more sensitive than the FA test and the FeLV p27 antisera provided in the Leukassay F (ELISA) test kits appear quite specific. The Leukassay is potentially able to detect limited or localized FeLV infection not involving the bone marrow and thus negative by the FA test. Therefore, FA negative, Leukassay positive cats should not necessarily be removed to prevent spread of FeLV to other cats.

An in situ molecular hybridization technique was developed for detecting FeLV or RD-114 RNA in individual cells. By this technique, uninfected cat thymocytes expressed RD-114 RNA but no FeLV-related RNA and both RD-114 and FeLV-related RNA were detected in cat placentas.

Significance to Biomedical Research and the Program of the Institute:
This project should help define the feline transforming gene (src) and its protein product in naturally occurring and chemically induced feline cancer and determine the feasibility of immune prevention of cancer in cats as a model system.

Proposed Course: This contract terminated September 21, 1981.

Date Contract Initiated: September 22, 1978

Current Annual Funding: No funding in FY 81

STANFORD UNIVERSITY (NO1-CP9-1011)

Title: Isolation and Characterization of T Lymphoma Cells and Normal Cell Receptors for Thymotropic Murine Oncornaviruses

Contractor's Project Director: Dr. Irving L. Weissman

Project Officer (NCI): Dr. Garrett V. Keefer

Objectives: Develop methods for the isolation of T lymphoma receptors for T-MuLV, compare the receptors from several T lymphomas which bind the same T-MuLV and compare receptors from a single tumor having the capacity to bind several different T-MuLVs. Compare receptors with other known gene products expressed on the surface of murine lymphocytes, determine if T-MuLV binding is by recognition of the viral env gene products gp70 and p15E. Compare T lymphoma receptor specificity with peptide maps of the bound ligands (e.g., gp70).

Major Findings: During this twelve month period, efforts have concentrated on three main areas:

1. The contractor tested whether B cell leukemias bear specific retrovirus receptors, and if so, whether they are immunoglobulin in nature. They found that a B cell lymphoma (BCL) proliferates in vivo and in vitro only when in contact with a virus producing adherent cell which bears the characteristics of an antigen presenting cell. The virus receptor on these B cell lymphomas is indeed the immunoglobulin, and the saturation of virus receptors with virus leads to proliferation by the B cell lymphomas. Anti-idiotypic antibodies directed against the immunoglobulin's variable regions blocks virus binding, and also detects a subset of T cell lymphomas binding the same virus.

2. Using a T lymphoma which binds a virus to a high degree, the contractor has analyzed the shed/secreted product which binds to a virus in terms of its virus-binding specificity, its size on molecular exclusion chromatography, and its charge distribution on ion exchange chromatography. It was demonstrated that the major viral binding protein on reducing SDS gels is found at molecular weight of approximately 85,000, with a binding component at 65,000-28,000, perhaps a proteolytic breakdown product of the higher molecular weight protein.

3. The contractor has examined several related but distinct cloned spontaneous AKR thymic lymphomas, and has carried out virus binding studies on each with all of the cloned viruses produced. Each of these viruses binds its homologous lymphoma better than other lymphomas arising in the same strain of mice; therefore, it was demonstrated that the surface receptor system involved in the virus binding is indeed specific. Using cloned and DNA-sequenced viruses, the contractor has found that there is specificity in virus binding by the lymphomas producing each of these viruses--indicating that the nucleotide sequence changes clustered in

the 3' end (env-R-C) appear to be important in defining the viral specificities which specific T lymphomas recognize.

Significance to Biomedical Research and the Program of the Institute: It has been determined that the envelope protein of the virion is the virus receptor molecule; specific antibody to this protein may prevent development of cancer. However, the cooperating receptor molecule has not been identified. Information relevant to the initial virus-cell interaction is basic to determining the complete course of events leading to malignant transformation.

Proposed Course: Studies will continue on the nature of B cell leukemia retroviral receptors and the examination of several related but distinct cloned spontaneous AKR thymic lymphomas in regard to their binding properties.

Date Contract Initiated: December 15, 1978

Current Annual Funding: $91,100

STANFORD UNIVERSITY (NO1-CP9-1044)

Title: Virologic, Biologic and Immunologic Characterization of Hodgkin's Disease and Other Human Malignant Lymphomas

Contractor's Project Director: Dr. Henry Kaplan

Project Officer (NCI): Dr. Stuart A. Aaronson

Objectives: To collect Hodgkin's disease (HD) tissue and serological samples for study. To cultivate human lymphoma tissue in vitro and characterize the established cell lines and clones derived from them. To investigate cultures for presence of virus, and characterize any viruses detected. To characterize, immunologically, the cells in relation to the host autoimmune responses.

Major Findings: The gibbon type C (GaLV) viral genome has been cloned in a lambda phage vector and mapped with restriction endonucleases. Several fragments have been subcloned in the pBR322 plasmid vector and labeled by nick-translation for use as molecular hybridization probes.

A heterologous monospecific antibody to the p28 of SSV-1/SSAV competes for binding to the viral p28 with a monoclonal antibody, 3D7(2B9), raised against the type C virus produced by the SU-DHL-1 human histiocytic lymphoma cell line. After in vitro translation of SSV-1/SSAV RNA, or of size-fractionated mRNA from SSV-1/SSAV-infected cells, both antibodies precipitate 6065 kd gag gene translation products.

The SSV-1 genome was apparently rescued from the HF/SSV-NP$_1$ nonproducer transformed marmoset cell line by cocultivation with SU-DHL-1 human histiocytic lymphoma cells or superinfection with SU-DHL-1 virus.

Sustained elevation of antibody titers to SU-DHL-1 viral proteins was observed in 2 young adult rhesus monkeys inoculated via the femoral bone marrow with SU-DHL-1 virus.

Two new permanent cell lines, SU-DLL-1 and SU-DUL-5, have been established from a lymphocytic lymphoma and an undifferentiated lymphoma, respectively. T-cell lymphomas have been maintained in long-term culture with the aid of T-cell growth factor. Several long-term cultures of the giant cells of Hodgkin's disease have been maintained for periods up to 6 months, but no permanent cell lines have been obtained. In vitro culture conditions have been developed for the long-term cultivation of human bone marrow with the sustained production of colony forming cells for periods in excess of 20 weeks.

The SU-HD-1 cell line has been found to release a lymphocyte mitogen with characteristics similar to those of lymphocyte activating factor (LAF, interleukin 1) after stimulation with neuraminidase-galactose oxidase or phorbol ester.

The Hodgkin's disease serum inhibitor of T-cell function has been partially characterized by thin layer chromatography and found to be a polar lipid.

Significance to Biomedical Research and the Program of the Institute: Hodgkin's disease has the attributes of an infectious process. This project focuses available technology to detect evidence for a virus etiology in this malignancy These studies may provide new information applicable to diagnosis, prognosis and treatment of Hodgkin's disease.

Proposed Course: This contract terminated June 15, 1981.

Date Contract Initiated: April 1, 1974

Current Annual Funding: $135,000 (six month extension)

TEL-AVIV UNIVERSITY (NO1-CP7-1011)

Title: Immunization of Mice with Purified MuMTV Polypeptides

Contractor's Project Director: Dr. Asher Frensdorff

Project Officer (NCI): Dr. Sandra Ruscetti

Objectives: To study the cellular and humoral responses of mice to MuMTV polypeptides and to immunize mice against spontaneous mammary neoplasia with purified MuMTV structural proteins.

Major Findings: Investigations of the humoral immune response of mice to MuMTV and MuMTV antigens, as they are presented to the immune recognition system of the natural host during normal ontogeny, i.e., on the membrane of virus-expressing cells, are continuing. For this purpose, young mice of RIIIf, C3Hf, BALB/c and C57B1 strains were injected with live spleen-cell suspensions from RIIIf, RIII, C3Hf, C3H, BALB/c or BALB/cfC3H mice. The only combination which crossed histocompatibility barriers was the immunization of C57B1 mice. Although such vaccination protocols have no practical application in vaccination programs, they are nevertheless expected to shed more light on the interrelation between antigen conformation and the resulting humoral response, as well as providing a more sensitive assay for endogenous virus expression than those commonly used. Results indicate that there is indeed a difference in the fine specificity of the antibodies formed against MuMTV when presented on cell surfaces (these

antibodies are predominantly anti-gp52) whereas vaccination with purified gp52 or with whole MuMTV induces a predominantly anti-gp36 response.

Significance to Biomedical Research and the Program of the Institute:
The use of vaccines with structurally intact viruses can be dangerous since potentially oncogenic materials can be transmitted as immunogens. This project will explore whether beneficial effects can be achieved from a safe vaccine which does not contain infectious nucelic acids.

Proposed Course: This contract terminated February 26, 1981.

Date Contract Initiated: September 9, 1977

Current Annual Level: No funding in FY 81

SUMMARY REPORT

RESEARCH RESOURCES

The Research Resources component of the Biological Carcinogenesis Branch (BCB) is responsible for planning, initiating and maintaining a coordinated program to anticipate and meet the needs of extramural investigators funded by the Branch as well as other investigators in the area of cancer research for research resources and logistical support. This coordinated program includes initiation, development, maintenance and management of resource contracts and the responsibility for the day-to-day general management and direction of all resources distribution.

Laboratory investigations carried out under the sponsorship of the BCB depend on the availability of adequate quantities of viruses, viral reagents, antisera, animals and clinical and laboratory materials of optimal purity, viability and potency, some of which are not available from the commercial sector. Research Resources provides these research materials and other supporting activities through contract operations representing four general areas. These include: activities directed toward production, characterization and distribution of purified viruses, viral reagents and appropriate antisera; activities concerned with animal resources, including production of pathogen-free species of animals, breeding of cotton-topped marmosets, maintenance of animal colonies including primates, and containment-type primate holding facilities; activities directed toward the production of specialized testing services for the examination of experimental materials; activities concerned with acquisition, collection, storage, inventory and distribution of normal and malignant human specimens.

During this report period virus production and antisera preparation efforts were shared by a total of six contracts whose funding represented 49% of the total Resources budget. The animal resources area accounted for 23% of the budget, provision of testing and service efforts accounted for 26% of the budget and human specimen acquisition and distribution accounted for the remaining 2%. (See Table I)

During the year, Research Resources has coordinated the distribution of a wide variety of biological resources and services to both NCI intramural investigators and qualified extramural investigators. In addition, at the request of the Director, NCI, Research Resources has performed user surveys on a number of resource contracts. In these surveys, recipients of the goods or services provided by a number of resource contracts were queried by letter and their evaluations of the products or services they received, as well as their estimates of the future needs for these products or services, were sought. The results of these user surveys were forwarded to the Director of the National Cancer Institute.

Based on the formal user surveys, as well as on "informal surveys" (conducted by telephone) and on the patterns of requests for various materials, several resource efforts are being cut back or eliminated. For example, a substantial decrease in the demand for Rous sarcoma virus, Prague C strain, and for murine xenotropic viruses has been noted in this reporting period. The production of these agents will not be continued in the future. In addition,

it was found that a very small portion of the utilization of a contract which provides serodiagnostic services for murine viruses was being utilized by the extramural research community. Accordingly, a decision was made that this effort will not be continued in the future and that those few investigators who need to acquire these services can do so through normal commercial channels. Based on the results of the formal user surveys, decisions were made to continue the preparation and distribution of antisera to oncogenic viruses and to continue to provide specific pathogen free White Leghorn chickens and Japanese quail.

In addition to these activities, Research Resources has coordinated the distribution of a variety of resources to Russian, French and Japanese scientists in keeping with formal U.S. or NCI international agreements with these countries covering the mutual exchange of cancer research materials. Materials supplied include purified and concentrated viruses, specialized viral proteins and antigens, normal and infected tissue culture cell lines, and a wide range of antisera and substantial amounts of AMV reverse transcriptase enzyme.

Another significant factor associated with the operation of the Research Resources program in this reporting period is the initiation of the system whereby the recipients of resource materials pay for the resources which they receive. Under this system, recipients of resources will reimburse the contractor for the costs of the materials and for the shipping costs. The contractor in turn will credit these proceeds against the monthly vouchers which he submits to the Government for payment under the contract. The Government then functions as a guarantor of a certain level of business or distribution activity rather than as the ultimate consumer of the resources distributed. This system, called the "payback" system will be initiated on May 19, 1981, with the inception of the new contract for the production and distribution of avian myeloblastosis virus and AMV reverse transcriptase and will be applied to other virus production and animal resource contracts as circumstances warrant.

TABLE I

Resource Activities by Type of Effort and Funding

Activity	Contracts	Funding
Viruses and Reagents	6	$3,206,158
Animal Resources	7	1,496,282
Testing and Service Effort	6	1,733,238
Human Specimens	1	145,000
	20	$6,580,678

RESEARCH RESOURCES

CONTRACT INDEX

Contract	Title	Page
Becton Dickinson and Co. NO1-CP-91004	Preparation of Antisera to Oncogenic or Potentially Oncogenic Viruses	1361
Child Research Center of Michigan NO1-CP-91003	Inter- and Intraspecies Identification of Cell Cultures	1361
Electro-Nucleonics Laboratories, Inc. NO1-CP-91001	Large Scale Production of Oncogenic or Potentially Oncogenic Viruses	1362
Electro-Nucleonics Laboratories, Inc. NO1-CP-01009	Production of RNA Avian Oncogenic Viruses	1363
Emory University NO1-CP-33343	Maintenance of an Irradiated Monkey Colony	1364
Hektoen Institute for Medical Res. NO1-CP-43344	Supply of Fresh Human Materials Obtained from Patients with Neoplastic Diseases	1365
Life Sciences, Inc. NO1-CP-33291	Production of Avian Myeloblastosis Virus and AMV Reverse Transcriptase	1366
Life Sciences, Inc. NO1-CP-61005	Production and Maintenance of Selected Reagent Grade-SPF Animals	1367
Life Sciences, Inc. NO1-CP-81023	Production, Purification and Concentration of Potentially Oncogenic Viruses	1368
Litton Bionetics, Inc. NO1-CP-91022	Operation of a Facility to Provide and Maintain Nonhuman Primates for Cancer Research	1368
Mason Research Institute NO1-CP-61052	Role of Hormonal Factors on Induction of Mammary Tumors in M-PMV Infected Animals	1369
Mason Research Institute NO1-CP-71001	Resources and Information Data Management.	1370

Meloy Laboratories NO1-CP-01020	Large Scale Tissue Culture Virus Production for Cancer Research	1371
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Microbiological Associates, Inc. NO1-CP-33288	Development of Lab Animal Virus Diagnostic Reagents and Operation of a Service Lab	1372
Microbiological Associates, Inc. NO1-CP-91016 & NO1-CP-11000	Operation of a Repository and Distribution Center for Biological Materials	1373
Navy, Department of YO1-CP-80500	Development and Characterization of Cell Substrates for Utilization in Cancer Research	1374
Rush-Presby.-St. Luke's Medical Ctr. NO1-CP-71014	Marmoset Colony for Cancer Research	1375
Sloan-Kettering Inst. for Cancer Res. NO1-CP-71003	Influence of Virus-Related Genes on Susceptibility to Cancer	1376

CONTRACT NARRATIVES

RESEARCH RESOURCES

Dr. John S. Cole, III (Acting)

BECTON-DICKINSON AND COMPANY (NO1-CP9-1004)

Title: Preparation of Antisera to Oncogenic or Potentially Oncogenic Viruses.

Contractor's Project Director: Dr. Roger Wilsnack

Project Officer (NCI): Dr. John S. Cole, III

Objectives: The objectives of this contract are to develop, produce, characterize and distribute antisera to oncogenic viruses and their components for use in the biological carcinogenesis program. In addition, antisera to immunoglobulins of various animal species and the T-antigen of SV40 are produced.

Major Findings: During the contract year, 300 reagent shipments, consisting of 116 different types of immunoreagents, were prepared and delivered to 220 investigators.

New procedures were adapted and modified for the preparation of autologous cell culture retrovirus antisera particularly suitable for immune precipitation. In addition, virus-induced isogeneic tumor systems have been maintained to provide antisera (free of antibody to extraneous proteins) to a variety of ecotropic and xenotropic viruses.

Significance to Biomedical Research and the Program of the Institute: The contractor serves as a centralized source of potent and specific antisera developed for use in cancer research. The close collaboration of the project with BCB research programs results in significant usefulness not only to Program but to the entire research community.

Proposed Course: This effort will continue to provide antisera for the needs of cancer-oriented investigators.

Date Contract Initiated: October 1, 1978

Current Annual Level: $528,704

CHILD RESEARCH CENTER OF MICHIGAN (NO1-CP9-1003)

Title: Inter-and Intraspecies Identification of Cancer Cells In Vitro

Contractor's Project Director: Dr. Ward D. Peterson, Jr.

Project Officer (NCI): Dr. John S. Cole, III

Objectives: This contract provides collaborating investigators of the Biological Carcinogenesis Branch (BCB) and members of the general scientific community with a service for rapid establishment or confirmation of species identity of cell culture systems.

Major Findings: During this report period, 150 cell cultures were received and characterized by 405 separate tests that utilized genetic markers of three different kinds; i.e., species membrane antigens, isozymes, and chromosome analysis. Nine cell species were encountered in the cell cultures received with approximately 30 percent of cultures being of human origin. Overall, 76 percent of the cultures were confirmed by characterization studies as being of the species of origin designated by the submitting investigator. The species of 16 cell lines either unknown or undesignated by the submitting investigator was established. All but one of the cocultivated cultures contained both species of cells and no other. Thirty-six percent of the large number of human cell cultures received for study were found to be contaminated either by another species of cell or by other human cell lines.

Significance to Biomedical Research and the Program of the Institute: In studies on oncogenic viruses, many cell cultures from the same or different species are used concurrently, which offer frequent opportunities for cross contamination. In multiple-species tumor transplantations, the species derivation of induced tumors sometimes comes into question. Generally, the significance of virus presence in tissue cells, the ability to grow virus, or the validity of virus isolaton systems are all dependent upon the assurance of the identity of the cell cultures used.

Proposed Course: This effort will be subject to competitive continuation and the successful offeror will continue to provide a service for cell identification.

Date Contract Initiated: January 1, 1979

Current Annual Level: $154,254

ELECTRO-NUCLEONICS LABORATORIES, INC. (NO1-CP9-1001)

Title: Large-Scale Production of Oncogenic or Potentially Oncogenic Viruses

Contractor's Project Director: Mr. John Lemp, Jr.

Project Officer (NCI): Dr. John S. Cole, III

Objectives: To provide for the isolation, large-scale production, concentration, assay and distribution of murine endogenous and xenotropic oncogenic viruses. Production and quality control involve tissue culture, electron microscopy, immunology, and various biochemical/biophysical techniques.

Major Findings: During the past year, 3,555 liters of virus-containing fluids were harvested from several diverse tissue culture systems. As directed by the NCI project officer, concentrates of purified viruses, and cells were distributed to the Research Resources repository and to individual investigators involved in a variety of research projects. More than 50 shipments to 35 research groups throughout the world emphasized the flexibility, value and far-reaching scope of this resources program. The major products included endogenous viruses (ATS-124 and BC-194: BALB virus 1), and xenotropic viruses (BC 177: BALB virus 2, BC232: NIH, BC 232: NZB, Clone 35: NZB).

Cell lines yielding xenotropic viruses generally produce about one-tenth as many virions as those producing ecotropic viruses. Thus, since the xenotropic viruses now comprise 100% of the total requested production, considerably increased volumes of tissue culture fluids are required to maintain equivalent quantities of concentrated xenotropic viruses for the various recipient investigators.

Significance to Biomedical Research and the Program of the Institute: In order to carry out research on the biochemistry and biophysics of oncogenic animal viruses, it has been necessary to provide large quantities of concentrated virus. With the emphasis on cloned fragments of nucleic acids and recombinant DNA technology, needs for these products have greatly decreased.

Proposed Course: This contract will terminate January 7, 1982.

Date Contract Initiated: January 8, 1979

Current Annual Level: $608,886

ELECTRO-NUCLEONICS LABORATORIES, INC. (NO1-CPO-01009)

Title: Production of RNA Avian Oncogenic Viruses

Contractor's Project Director: Mr. John Lemp, Jr.

Project Officer (NCI): Dr. John S. Cole, III

Objectives: To produce, purify, characterize and distribute Rous sarcoma virus, Prague c strain (RSV-Pr. c) to collaborating investigators funded by the BCB. Production and quality control involve tissue culture, electron microscopy, immunology and various biochemical/biophysical techniques.

Major Findings: This contract has produced substantial amounts of Rous sarcoma virus by two different experimental protocols to provide materials high in either viral protein or intact high molecular weight nucleic acid. The product has been evaluated by several investigators and has been found to be of excellent quality.

Significance to Biomedical Research and the Program of the Institute: Large quantities of concentrated and well characterized virus have been

utilized to conduct research on the biophysics and biochemistry of oncogenic viruses. Changes in research directions and advances in technology have markedly decreased the needs for these items.

Proposed Course: This effort will terminate November 6, 1981

Date Contract Initiated: November 7, 1979

Current Annual Level: $481,500

EMORY UNIVERSITY, YERKES PRIMATE CENTER (NO1-CP3-3343)

Title: Maintenance of a Colony of Irradiated, Aging Rhesus Monkeys

Contractor's Project Director: Dr. Harold McClure

Project Officer (NCI): Dr. Garrett V. Keefer

Objectives: To monitor the incidence of tumors in a unique group of irradiated, aging rhesus monkeys and to supply tissue from tumors to the scientific community for transplantation, tissue culture, and virus isolation studies.

Major Findings: Triannual physical examinations have been conducted on each of the 52 rhesus monkeys included in this study. The periodic examinations concentrate on the detection of developing neoplasms. Most of the animals remain in fair to good physical condition. During the most recent survey, six animals were classified as moderately to severely emaciated; eight animals were noted to be relatively thin; and 38 animals were noted to be in fair to good physical condition. Skin and/or subcutaneous nodules or masses are present in 16 animals. Most of these consist of small papilloma-like lesions of the skin or small subcutaneous masses. Two animals have large skin or subcutaneous masses: a basal cell carcinoma of the skin of the upper arm and an adenosis of the parotid gland. Four animals have small or large masses in the lower abdomen in the region of the uterus. During the past year, one animal in this group died. The death was associated with squamous cell carcinoma.

Observations in this group of animals continue to confirm their value as a source of nonhuman primate tumor material, as 21 of 44 (47.7 percent) animals which have died have had neoplasms. In addition, 4 animals with neoplasms are currently present in the colony. Twenty-one of the 25 animals (84 percent) with tumors had a history of radiation exposure.

Significance to Biomedical Research and the Program of the Institute: This project provides tumor tissues and other important specimens from aging, irradiated subhuman primates for research sponsored by the BCB. Malignant changes in these irradiated primates may provide useful information which might be applied to humans, who are also subjected to similar physical stresses.

Proposed Course: The entire group of monkeys will continue to be monitored for neoplasia by physical and hematologic examinations until the expiration of the approved project plan on April 30, 1982. All tumors which develop will be evaluated by the contractor using light and electron microscopy. Specimens of these tumors will be made available to qualified investigators.

Date Contract Initiated: May 1, 1971

Current Annual Level: $33,000

HEKTOEN INSTITUTE FOR MEDICAL RESEARCH (NO1-CP4-3344)

Title: Supply of Fresh Human Materials Obtained from Patients with Neoplastic Diseases

Contractor's Project Director: Dr. Paul Szanto

Project Officer (NCI): Ms. Wilma L. Varrato

Objectives: The objective of this contract is to provide sterile, viable, and freshly frozen normal or neoplastic human specimens to the Biological Carcinogenesis Branch (BCB) for distribution to collaborating laboratories for biological, chemical, and virological cancer research investigations.

Major Findings: During the year, the contractor shipped 606 fresh viable specimens to the BCB resources processing center, and 120 fresh specimens directly to investigators. In addition, the contractor supplied 1,112 frozen tumor and organ specimens to the Research Resources repository. The tumors included a wide variety of carcinomas, adenocarcinomas, sarcomas, and metastatic tissues. Normal tissues and benign tumors were also provided.

Significance to Biomedical Research and the Program of the Institute: A continuous supply of appropriate human tissues from patients with cancer is vital to cooperative cancer research investigations. In order to carry out important studies on the biochemistry and immunology of suspected oncogenic human viruses, it is important that large quantities of human malignant tissues be available for analysis.

Proposed Course: This contractor continued to supply tissue specimens from patients with neoplastic disease and from normal individuals for the duration of the approved project plan, which expired December 16, 1980.

Date Contract Initiated: June 17, 1974

Current Annual Level: No funds in FY81

LIFE SCIENCES, INC. (NO1-CP3-3291 and NO1-CPO-1014)

Title: Production of Avian Myeloblastosis Virus and AMV Reverse Transcriptase

Contract's Project Director: Dr. Joseph W. Beard

Project Officer (NCI): Dr. John S. Cole, III

Objectives: The objectives of this project are the large-scale in vivo production of BAI strain A avian myeloblastosis virus (AMV) and the preparation and distribution of significant quantitites of AMV reverse transcriptase enzyme.

Major Findings: In accordance with the objectives of the contract, BAI strain A avian myeloblastosis virus has been produced by harvest of the agent from the blood plasma of chicks with myeloblastic leukemia induced by the agent. In the past year, a total of 336,265 chicks have yielded 755 g of virus, of which 100 g have been shipped to 26 investigators in America. A total of six shipments of 13.2 g were made to laboratories in several countries abroad (Canada, England, France, Germany, India, Russia, Sweden, and Switzerland). Virus was also used in the laboratory for passage of the disease (23 g) or preparation of reverse transcriptase (336 g). In the same interval, 208 ml of myeloblast cells of myeloblastic leukemia were sent to four workers in America and abroad. Purified reverse transcriptase continues to be isolated in yields of 20,000 -25,000 units per g (wet weight) of purified AMV, and a specific activity of 30,000 - 50,000 units/mg of protein. The isolation procedure was greatly simplified to produce preparations essentially free of RNase. A total of 9,141,940 units of the enzyme were sent to 587 investigators in the past 12 months, 5,997,200 units to 386 laboratories in the continental United States and 3,144,740 units were sent in shipments to laboratories in various countries abroad (Australia, Austria, Brazil, Bulgaria, Canada, Denmark, England, Finland, France, Germany, Hawaii, India, Israel, Italy, Japan, Mexico, Norway, Russia, Scotland, South Africa, Sweden, Switzerland, and The Netherlands).

Significance to Biomedical Research and the Program of the Institute: An important aspect of studies of biological carcinogenesis involves studies on the interaction of cDNA copies of oncornaviral genomes with cellular protein synthesis. Such studies could aid in determining and assigning functions to various parts of the viral genome and in determining the possible function of postulated "src" or "onc" genes. For these studies, a large and consistantly active supply of "reverse transcriptase" is vital. In addition to these areas, reverse transcriptase is of great importance to certain recombinant DNA studies. A third area of importance is the provision of large (multigram) quantities of avian myeloblastosis virus for studies on the mechanism of induction of avian tumors.

Proposed Course: This contract was subject to competitive continuation in FY 81. The incumbent, as the successful awardee will continue to meet requests for avian myeloblastosis virus and will continue the preparation and distribution of large quantities of AMV reverse transcriptase. Beginning with the next contract year, a charge is being made for the various

products distributed by the contractor.

Date Contract Initiated: April 19, 1971 and May 19, 1981

Current Annual Level: $522,354

LIFE SCIENCES, INC. (NO1-CP6-1005)

Title: Production and Maintenance of Selected Reagent Grade-Specific Pathogen-Free Animals

Contractor's Project Director: Dr. Wendall M. Farrow

Project Officer (NCI): Dr. John S. Cole, III

Objectives: To produce specific pathogen-free (SPF) animals for cancer research. SPF animals are maintained under environmentally controlled conditions which preclude intercurrent infection by pathogenic microorganisms or infestation by parasites and are referred to as "reagent-grade" hosts.

Major Findings: A small inbred SPF BALB/c colony, housed in an isolator, supplied 300 young breeders to expand one supply colony maintained in a barrier cubicle. One barrier-sustained outbred BALB/c colony supplied 260 time-bred animals to recipients. A caesarean-derived SPF BALB/c nude strain supplied 2,300 nu/nu, 1240 nu/+ mice and 126 time-bred mice to investigators. No detectable antibody to ten murine viruses was observed in the normal or nude BALB/c mouse colonies. A small expansion colony of NIH Swiss mice produced 300 time-bred animals and 140 adults for recipients. The NIH Swiss nude mouse colonies supplied 3,200 nu/nu, and 1,500 nu/+. The NIH Swiss strains continue to show no detectable antibody to common murine agents. A small maintenance colony (10 males, 50 females) of RIII mice was held in an isolator system.

An outbred SPF, leukosis-free flock of Japanese quail produced 18,160 fertile or embryonated eggs for investigators. A supply flock of avian pathogen-free White Leghorn chickens supplied 10,000 C/E, fertile eggs. Additionally, 3,000 embryonated eggs (9-11 days incubation) and 32 chicks 1-3 days of age were produced for recipients. To maintain definitive status of the White Leghorn flock, 100 single embryos were phenotyped and monitored for avian oncornavirus GS-1, chick helper factor and RIF. Monitoring of sera from retired pedigreed chickens indicated no evidence of avian pathogens, including avian leukosis and Marek's herpesvirus.

Significance to Biomedical Research and the Program of the Institute: This contract provides investigators funded by the Biological Carcinogenesis Branch genetically and microbiologically well-defined laboratory animals. The advantage of having such animals is that oncogenic and suspected oncogenic viruses can be administered to them with minimal danger of interference from other contaminating, adventitious microorganisms. Therefore, research can be carried out upon animals with a known and controlled viral flora, and cell lines can be derived from these animals which share this same advantage.

Proposed Course: Based on changing needs and the commercial availability of the mice supplied, only the avian portion of this service-type contract for the production of reagent grade SPF animals will be continued. Beginning with the next contract year, a charge is being made for the various products distributed by the contractor.

Date Contract Initiated: February 8, 1968

Current Annual Level: $422,032

LIFE SCIENCES, INC. (NO1-CP8-1023)

Title: Production, Purification, and Concentration of Potentially Oncogenic DNA Viruses

Contractor's Project Director: Dr. Meihan Nonoyama

Project Officer (NCI): Dr. John S. Cole, III

Objectives: The objectives of this contract are to prepare, process, and purify high quality Epstein-Barr virus (EBV) of both B95-8 and P3HRI strain, and to prepare 55S EBV DNA for biochemical/molecular biology studies of the role of EBV in human cancer.

Major Findings: During the past year, the contractor has produced and processed over 2,000 liters of EBV containing fluids. Approximately 8.0 X 10^9 transforming units of B-95 virus and 1.2 X 10^{10} early antigen inducing units of P3HR-1 were distributed. In addition, 600 µg of purified viral DNA, approximately 700 gm of B95 cell pelletes and 500 gm of Raji cells were distributed.

Significance to Biomedical Research and the Program of the Institute: Of all the viruses implicated in human cancer, perhaps the best case for a viral-tumor association can be made for EBV. It is of extreme importance to provide sufficient amounts of the infectious and transforming virus and of viral DNA so that studies can proceed to further elucidate the possible role of this agent in human disease.

Proposed Course: This contract will be subject to competitive continuation and the successful offeror will continue to provide high quality virus and viral products to qualified investigators.

Date Contract Intiated: July 31, 1978

Current Annual Level: $315,000

LITTON BIONETICS, INC. (NO1-CP9-1022)

Title: Operation of a Facility to Maintain Subhuman Primates for Cancer Research

Contractor's Project Director: Dr. John Cicmanec

Project Officer(s) (NCI): Dr. Garrett V. Keefer

Objectives: The objective of this contract is the maintenance of a laboratory necessary for inoculation, care, and monitoring of primates.

Major Findings: It is anticipated that this effort will continue to provide a biologically contained holding facility for several species of nonhuman primates inoculated with tumor viruses.

Significance to Biomedical Research and the Program of the Institute: Since over 170 nonhuman primates are currently involved in ongoing studies on the effects of oncogenic or suspected oncogenic agents, it is necessary to have a facility for the contained housing and observation of these animals.

Proposed Course: This project will continue to provide long-term holding and study of experimental animals inoculated by investigators.

Date Contract Initiated: January 1, 1979

Current Annual Level: $404,326

MASON RESEARCH INSTITUTE (NO1-CP6-1052)

Title: Studies on the Role of Hormonal Factors in the Induction of Mammary Tumors in Rhesus Monkeys Infected with Mason-Pfizer Monkey Virus

Contractor's Project Director: Dr. Arthur E. Bogden

Project Officer (NCI): Dr. Garrett Keefer

Objectives: To provide support for the holding and observation of virus infected nonhuman primates.

Major Findings: MPMV, originally isolated from a spontaneous breast tumor in a rhesus monkey, has been described as a prototype virus of the type D retroviruses. Active infection in the majority of MPMV-inoculated subhuman primates being held at the contractor's laboratory and monitored for mammary tumor induction has been confirmed by radioimmunoassay screening for the specific MPMV core protein p27 in the serum. Emerging parallels in the immunobiological characteristics of MuMTV and MPMV suggest a possible role of MPMV in mammary tumorigenesis in the subhuman primate.

Significance to Biomedical Research and the Program of the Institute: MPMV was recovered from a primate mammary cancer; it possesses characteristics common to known oncogenic viruses, and cross-reactions have been observed between the viral antigens and antigens present in human breast cancer specimens. Investigation for possible oncogenic properties in a primate host is valuable as a potential model for human breast cancer.

Proposed Course: Continuation of the holding and observation of the primates until the expiration of the approved project plan (June 6, 1982) is planned to provide information on the role of MPMV in mammary tumorigenesis in subhuman primates.

Date Contract Initiated: June 9, 1979

Current Annual Level: $140,250

MASON RESEARCH INSTITUTE (NO1-CP7-1001)

Title: Resources and Information Data Management

Contractor's Project Director: Mr. Mark Gladstone

Project Officer (NCI): Ms. Wilma Varrato

Objectives: To assist in processing, storage, and retrieval of data associated with research resource materials of the Biological Carcinogenesis Branch.

Major Findings: During the last year, the contractor continued to provide computer support to the Biological Carcinogenesis Branch. Efforts were directed toward: a) the design and development of new and revised systems for the management of the collection, storage, and distribution of research materials; b) data entry to support the operation of existing systems; c) the development and production of reports systems; and d) documentation of revisions to current and newly developed systems and programs.

Significance to Biomedical Research and the Program of the Institute: Computerization of resources data makes it possible for the Biological Carcinogenesis Branch to exercise close control over the inventory of viruses, sera, human tissues, and other materials provided by the NCI and used in cancer research. In addition, computerization makes it possible to rapidly obtain information necessary to determine availability, location, quantity, etc. of all resources within its jurisdiction; thereby, permitting rapid response to needs of the Program while avoiding resource excesses or shortages.

Proposed Course: This effort will be subject to competitive selection in the current year. The successful offeror will continue to provide programming, systems analysis and data manipulation to the Biological Carcinogenesis Branch.

Date Contract Initiated: March 1, 1977

Current Annual Level: $351,288

MELOY LABORATORIES, INC. NO1-CPO-1020

Title: Large Scale Tissue Culture Virus Production For Cancer Research

Contractor's Project Director: Dr. George Gray

Project Officer (NCI): Dr. John S. Cole, III

Objectives: The objectives of this partial successor to contract NO1-CP-91018 are to provide for the large-scale production and distribution of viruses of continuing interest to qualified investigators. These viruses include RD114 virus, Baboon Endogneous virus, Mason-Pfizer monkey virus, and the mouse mammary tumor virus in the C3H, GR and RIII cell systems.

Major Findings: This new effort is determining the optimum growth conditions for the production of high quality viral products and determining quality control parameters on current production lots. Production lots are being evaluated by the scientific community.

Significance to Biomedical Research and the Program of the Institute: This production effort for type B, C and D retroviruses provides sufficient quality of these agents for ongoing and planned research activities carried out by qualified investigators.

Proposed Course: To continue the production and distribution of these agents to the extent they are needed by investigators.

Date Contract Initiated: September 24, 1980

Current Annual Level: $693,434

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (NO1-CP6-1038)

Title: Acquisition of Human Specimens For Use in Cancer Research

Contractor's Project Director: Dr. Yashar Hirshaut

Project Officer (NCI): Dr. Jack Gruber

Objectives: To collect sera and tissues from human subjects with neoplastic tumors to be used in cancer research studies.

Major Findings: The Tumor Procurement Center at Memorial Hospital for Cancer and Allied Diseases is a major facility for the collection and distribution of specimens used in cancer research. During the last 12 month period, a total of 9,842 human specimens were collected, including 5,271 tissues and 4,571 sera. A total of 11,800 specimens were sent to 128 investigators.

Significance to Biomedical Research and the Program of the Institute: This contract provides fresh and frozen human specimens (tissue and sera)

obtained as a by-product of normal surgical procedures for use in cancer research.

Proposed Course: This contract terminates on February 28, 1982.

Date Contract Initiated: March 1, 1978

Current Annual Level: $145,000

MICROBIOLOGICAL ASSOCIATES, INC. (NO1-CP3-3288)

Title: Development of Laboratory Animal Virus Diagnostic Reagents and Operation of a Service Laboratory

Contractor's Project Director: Dr. Michael Collins

Project Officer (NCI): Dr. John S. Cole, III

Objectives: To develop reagents and tests for the detection of rodent viruses; to apply these and other tools in the determination of the importance of the indigenous viruses in experimental systems; to study means for elimination of viruses from laboratory animal populations; and to assist in the characterization of the gene-dependent expression of murine leukemia.

Major Findings: The contractor operates a murine virus serodiagnostic and viral diagnostic laboratory for the Biological Carcinogenesis Branch, NCI. During this report period, over 49,000 serological tests were performed on sera from mice, rats, hamsters or guinea pigs. Many of the tests were in conjunction with the NIH ectromelia virus outbreak. A total of 164 animal tissues, transplantable tumors, ascites, cell cultures, and viral reagents were tested for murine viral contamination by the mouse antibody production (MAP) procedure. Special tests for the detection of lactic dehydrogenase (LDH) virus were conducted on 211 tumor or oncogenic viral preparations and 3,120 XC plaque assays were conducted for the detection of murine leukemia in cell cultures and animal tissues. The contract produced and maintained an inventory of 36 different viral diagnostic reagents. Production included 3,500 ml of viral reagents and 100 ml of specific antisera. These reagents are available and were supplied on request to 20 investigators. Direct isolations of ectromelia and Sendai viruses from tissues of diseased animals or from cell cultures were conducted on 229 specimens.

The contract continues to provide a service, which is not available elsewhere, to several NIH programs involving (1) the effects of host genetics on the expression of murine leukemia; (2) the pathogenicity of murine type C virus isolates; (3) the infectivity of polyoma virus DNA; and (4) type C RNA virus isolates from wild mice.

Significance to Biomedical Research and the Program of the Institute: These virus diagnostic capabilities provide the NCI with the ability to monitor laboratory rodent colonies and laboratory animal-produced viral reagents and tumors which have resulted in the production of highly

characterized systems for cancer research. This contract provides assistance and guidance of particular importance for the detection of lymphocytic choriomeningitis (LCM) in rodent systems.

Proposed Course: Due to the very low utilization of these services by DCCP funded investigators (less than 10% of the total contract) and the commercial availability of many of these services, this contract has been extended for a terminal year to allow other NIH Institutes to determine appropriate mechanisms for the possible continuation of this effort.

Date Contract Initiated: April 10, 1961

Current Annual Level: $425,166

MICROBIOLOGICAL ASSOCIATES, INC. (NO1-CP9-1016 and NO1-CP1-1000)

Title: Operation of a Repository and Distribution Center for Biological Materials

Contractor's Project Director: Ms. Cynthia McKinney

Project Officer (NCI): Dr. John S. Cole, III

Objectives: This contract provides a secure low-temperature storage facility for biological reagents and clinical specimens prepared for the BCB. The facility receives, inventories, stores, and distributes these materials to both domestic and foreign recipients as authorized by the NCI Project Officer. Accurate computerized inventories and records of shipments are provided to the Branch.

Major Findings: This effort made over 97 shipments to domestic laboratories and 22 shipments to foreign laboratories of viral reagents and antisera in the past year. The contractor received 88 shipments, consisting primarily of 360 lots of viral reagents and approximately 2,600 human specimens. Over 5000 human specimens were distributed during the past year.

Significance to Biomedical Research and the Program of the Institute: The storage and shipping facilities operated under this contract enable grantees and contractors of the BCB to have access to a large inventory of special research materials.

Proposed Course: To continue the operation of a repository for viruses, viral products and human specimens.

Date Contract Initiated: March 1, 1981

Current Annual Level: $300,752

NAVY, DEPARTMENT OF (YO1-CP8-0500)

Title: Development and Characterization of Cell Substrates for Utilization in Cancer Research and Allied Studies

Contractor's Project Director(s): Dr. Neylan Vedros
Dr. Walter Nelson-Rees

Project Officer (NCI): Dr. Jack Gruber

Objectives: The Cell Culture Laboratory (CCL) is physically located at the Naval Biosciences Laboratory (NBL), Oakland, California. This project includes the developing and evaluating of cell substrates for the study of cancer viruses; developing large quantities of specific cell substrates; karyotyping of cell cultures; and performing biophysical, virological, and cytogenetic applied research.

Major Findings: During the reporting period, the contractor continued to initiate, grow, preserve, characterize, and distribute a variety of human and animal cells for utilization in cancer research. Procedures utilized are such that antibiotic-free cultivation is achieved in the majority of cases. Routine laboratory procedures are such that mycoplasma contamination occurs at only a very low frequency among the numerous cultures initiated, grown, and preserved in this laboratory.

During this reporting period, approximately 1,200 cell culture seed stocks (ampoules and flasks) were distributed to 200 individual scientists. A number of valuable cell lines of tumor origin have been received from other laboratories and processed at CCL for distribution; among these were four of human origin. Approximately 20 laboratories have submitted 100 individual cell cultures for analysis of species and cell line purity. Approximately 62 percent were as purported and others involved intra- and inter-species contamination. Activities of this nature are projected to continue at the same or increased levels for the duration of the contract.

Morphologically abnormal cell lines are being compared to morphologically normal cells in terms of growth pattern, cell doubling time, saturation density, clonal growth on various substrates, karyology, and tumorgenicity in nude mice. Clonal growth studies are in progress on a variety of low passage, non-HeLa, human cancer cell lines in an effort to define the optimum nutrition and environmental conditions for culture of such cells.

Karyologic characterization of all cells maintained in the repository continues and specific collaborative programs requiring the use of karyologic data are in progress.

Significance to Biomedical Research and the Program of the Institute: The contractor has an excellent tissue culture facility and is supplying cell cultures for cancer research studies to NCI investigators, to BCB contractors and grantees, and to the general scientific community. The contract continues to develop techniques for the identification and study of cells oriented toward a study of the fundamental biology of tumor cells and the interaction between tumor cells and viruses of oncogenic importance.

Proposed Course: To continue the operation of a repository and distribution center for cell cultures.

Date Contract Initiated: October 1, 1980

Current Annual Level: $480,000

RUSH-PRESBYTERIAN-ST. LUKE'S MEDICAL CENTER (NO1-CP7-1014)

Title: Marmoset Colony for Cancer Research

Contractor's Project Director: Dr. Lauren G. Wolfe

Project Officer (NCI): Dr. Garrett V. Keefer

Objectives: The aim of this contract is the development and maintenance of a marmoset breeding colony in order to ultimately have appropriate numbers of these animals available for experimental use, and to provide a marmoset facility that includes a support laboratory for the inoculation and monitoring of animals under study as well as an adequate animal containment-holding area.

Major Findings: This program provides a resource of marmosets and the technical expertise for performance of experimental studies. The marmoset colony currently numbers 383 animals: 182 breeders, 80 experimental animals, and 113 young uninoculated animals. The breeding colony contains 70 cotton-topped (Saguinus oedipus), 98 white-lipped (Saguinus sp.) and 14 common (Callithrix jacchus) marmosets. Seventy-seven offspring were produced during the reporting period. The colony served as a resource in support of six research projects approved by the Research Resources Primate Utilization Review Group. Eighty-eight animals were used in the experimental studies; virology, clinical pathology and pathology services were supplied for performance of certain studies.

Significance to Biomedical Research and the Program of the Institute: Inasmuch as experimentation for the biological activity of candidate human viruses will not be carried out on humans, it is imperative that another system be developed for these determinations and subsequently for the evaluation of vaccines or other measures of control. The close phylogenetic relationship of the lower primates to man justifies utilization of these animals for these purposes. The marmoset appears to be especially suitable for use as a comparative model system. To date, at least five and possibly six virus tumor models, including Epstein-Barr and Herpesvirus saimiri viruses, have been established in marmoset monkeys. In addition, because of its small size, the marmoset is economical to house yet it is large enough for routine surgical procedures and serological monitoring.

Proposed Course: Continuation of support services as described.

Date Contract Initiated: April 1, 1977

Current Annual Level: $299,639

SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH (NO1-CP7-1003)

Title: Influence of Virus-Related Genes on Susceptibility to Cancer

Contractor's Project Director: Dr. Edward A. Boyse

Project Officer (NCI): Dr. Stephen O'Brien

Objectives: (1) Breeding, maintenance, and supply of congenic strains of mice which differ from their partner strains in expression of genes concerned with production or control of leukemia virus, and (2) testing these paired strains for their susceptibility to spontaneous and induced malignancy.

Major Findings: During the past year, the contractor continued to supply breeding pairs of congenic mice to both foreign and domestic grantees and contractors of the BCB. More than 40 percent of the recipients have published papers utilizing the progeny of these animals. Efforts continue to be directed to attaining the 20 generations of mice necessary to a defined genetic background.

Significance for Biomedical Research and the Program of the Institute: Genetic control of susceptibility to spontaneous and viral-induced leukemia in mice has been well documented. However, the mechanism of control by the several loci involved has only recently received attention. The present study has been designed to assign specific functions to each controlling genetic allele. Identification of genetic control mechanisms in murine strains should form the groundwork for identifying similar controlling factors in other species, including man.

Proposed Course: "Quartets" of four strains, representing two base strains and two corresponding congenic strains in which the differentiating alleles have been switched both ways, will be developed and supplied to all qualified, interested investigators. Seven quartets are planned covering the H-2, Fv-1, PC, TL and GIX loci. The influence of each of the alleles on expression of virus, host response to viral products, and occurrence of leukemia will be investigated by monitoring virological, immunological and pathological parameters during the life span of these strains.

Making use of congenic stocks already established, selected 'double' congenic strains will be made in which alleles of two loci will be substituted on a genotypic background common to that on which each allele was individually isolated in the first place. This will permit assessment of the joint action of alleles of two genes, as compared with either alone, on virus-related characteristics and on susceptibility to neoplasia and other diseases associated with type C virus.

Date Contract Initiated: December 15, 1980

Current Annual Level: $140,000

BIOLOGICAL CARCINOGENESIS BRANCH

GRANTS ACTIVE DURING FY81

DNA VIRUS STUDIES

Investigator/Institution/Grant Number	Title
ALONI, Yosef Weizmann Institute of Science 5 RO1 CA 14995-08	Control of Gene Expression in Tumor Viruses and Cells
ALWINE, James C University of Pennsylvania 5 RO1 CA 28379-02	Regulation of DNA Tumor Virus Gene Expression
BASILICO, Claudio New York University 5 RO1 CA 11893-11	Cellular and Viral Control of Oncogenetic Transformation
BASILICO, Claudio New York University 2 PO1 CA 16239-08	Biosynthesis in Normal and Virus-Transformed Cells
BAUM, Stephen Yeshiva University 5 RO1 CA 10945-11	Interaction of Oncogenic Viruses (Adenovirus and SV40)
BENJAMIN, Thomas L Harvard University 5 RO1 CA 19567-05	Mechanism of Cell Transformation by Polyoma Virus
BENJAMIN, Thomas L Harvard University 2 RO1 CA 25390-03	Effects of HR-T Mutations on Polyoma Gene Expression
BERG, Paul Stanford University 5 RO1 CA 15513-08	Basic Mechanisms in Viral Carcinogenesis
BERK, Arnold J University of California (LA) 5 RO1 CA 25235-03	Biosynthesis of Adenovirus Early RNAs
BLACK, Paul H Boston University 5 RO1 CA 28107-03	Mechanisms of Transformation by Oncogenic Viruses
BOTCHAN, Michael R University of California (Berkeley) 1 RO1 CA 30490-01	Transformation of Cells by SV40 Virus

BROCKMAN, William W
University of Michigan
5 R01 CA 19816-05

Role of SV40 Gene A in Cellular Transformation

BUTEL, Janet S
Baylor College of Medicine
2 R01 CA 22555-05

Biological Properties of SV40 Early Proteins

BUTEL, Janet S
Baylor College of Medicine
5 R01 CA 25215-03

Tumor Virus Effects on Mouse Mammary Epithelial Cells

CALNEK, Bruce W
Cornell University
5 R01 CA 06709-20

Avian Leukosis Complex

CARROLL, Dana
University of Utah
5 R01 CA 26128-02

A Novel Function of the Simian Virus 40 Genome

CARROLL, Robert B
New York University
5 R01 CA 20802-05

Biochemical and Functional Properties of SV40 T Antigen

CARTER, Timothy H
St. John's University
5 R01 CA 25757-04

The Regulation of Adenovirus Gene Expression

CLOUGH, Wendy G
University of Southern California
5 R01 CA 23070-05

EBV DNA Synthesis in Transformed Lymphocytes

CONSIGLI, Richard A
Kansas State University
3 R01 CA 07139-18S1

Studies in Polyoma Transformed Cells--Virion Proteins

COOPER, Neil R
Scripps Clinic
2 R01 CA 14692-09

Humoral Immunity to Viruses and Virus-Infected Cells

COURTNEY, Richard J
University of Tennessee
5 R01 CA 27870-02

Proteins of HSV-Infected and Transformed Cells

CROCE, Carlo M
Wistar Inst. of Anatomy and Biology
5 R01 CA 16685-06

Mapping of Tumor Virus Genomes in Transformed Cells

DANNA, Kathleen J
University of Colorado (Boulder)
5 R01 CA 24924-03

SV40 Early Proteins--Possible Roles in Oncogenesis

DARNELL, James E, Jr Rockefeller University 5 RO1 CA 16006-08	RNA and Growth Control in Animal Cells
DE MARCHI, Jeanette M Vanderbilt University 2 RO1 CA 20806-04	Induction by Cytomegalovirus of Cell DNA Synthesis
DE PAMPHILIS, Melvin L Harvard University 5 RO1 CA 15579-08	Tumor Virus DNA Replication: A Probe into Oncogenesis
DIAMANDOPOULOS, George T Harvard University 5 RO1 CA 08731-15	Viral Carcinogenesis with Special Reference to SV40
DI MAYORCA, Giampiero College of Medicine & Dentistry, NJ 5 RO1 CA 25168-02	Transformation Genes of Simian Virus 40
DI MAYORCA, Giampiero College of Medicine & Dentistry, NJ 5 RO1 CA 25169-02	BK Virus, A Human Papovavirus
ECKHART, Walter Salk Institute for Biological Studies 5 RO1 CA 13884-10	Viral Gene Functions and Regulation of Cell Growth
EGGERDING, Faye A University of California (LA) 5 RO1 CA 25545-02	Regulation of Adenovirus 2 Transcription
EVANS, Mary J N Y State Department of Health 5 RO1 CA 22655-03	DNA Polymerase(s) of the Replication SV40 Chromosomes
FALK, Lawrence A, Jr Harvard University 5 RO1 CA 27225-03	Study of Human and Simian Lymphotropic Herpesviruses
FAREED, George C University of California (LA) 5 RO1 CA 20794-05	Simian Virus 40, DNA Replication and Transformation
FLUCK, Michele M Michigan State University 5 R23 CA 27453-02	The Role of Two Polyoma Early Genes in Transformation
FLUCK, Michele M Michigan State University 1 RO1 CA 29270-01	Control of Gene Expression on Viral Transformants

FOLK, William R University of Michigan 5 R01 CA 13978-09	Mammalian Cell Transformation by Oncogenic Viruses
FRENKEL, Gerald D Albany Medical College 5 R01 CA 22965-04	Inhibition of Cellular DNAses by DNA Tumor Viruses
FRIEDMANN, Theodore University of California (San Diego) 5 R01 CA 24288-03	Nucleotide Sequencing of Polyoma DNA
GALLOWAY, Denise A Fred Hutchinson Cancer Research Ctr 5 R01 CA 26001-02	Herpesvirus Expression in Transformation and Latency
GAYNOR, Richard B University of California (LA) 1 R23 CA 30981-01	Adenovirus 5 Mutants in Transforming Functions
GINGERAS, Thomas R Cold Spring Harbor Laboratory 3 R01 CA 27275-01S1	DNA Sequence and Computer Analysis of a Tumor Virus
GLASER, Ronald Ohio State University 5 R01 CA 23807-03	Expression of Epstein-Barr Virus in Hybrid Cells
GLASER, Ronald Ohio State University 1 R01 CA 29066-01	Epstein-Barr Virus DNA In Transfected Cells
GRALLA, Jay University of California (LA) 5 R01 CA 19941-05	Regulation of Transcription by DNA-Protein Complexes
GREEN, Maurice St. Louis University 5 R01 CA 28689-02	Human Papillomaviruses
GREEN, Maurice St. Louis University 5 R01 CA 29561-24	Biochemistry of Animal Virus Multiplication
GREEN, Melvin H University of California (San Diego) 5 R01 CA 24281-03	The Process and Control of Transcription of SV40
GUTAI, Mary W New York State Department of Health 5 R01 CA 28250-02	SV40 DNA Replication and Recombination in Animal Cells

HAGER, Lowell P University of Illinois (Urbana) 5 R01 CA 17619-06	Biochemical Studies on T Antigen and Transformed Cells
HALLICK, Lesley M University of Oregon Hlth. Sci. Ctr. 5 R01 CA 24799-02	Replication and Repair of Viral DNA and RNA Complexes
HARTER, Marian L College of Medicine & Dentistry, NJ 5 R01 CA 28414-02	Functions of Early Proteins Encoded by Adenovirus
HAYWARD, Gary S Johns Hopkins University 5 R01 CA 22130-04	Structural Organization of Herpes Virus DNA Molecules
HAYWARD, Gary S. Johns Hopkins University 5 R01 CA 28473-02	Cellular Transformation by DNA of Human Herpesviruses
HELD, William A New York State Dept. Health 5 R01 CA 27647-02	TK-Mutants of Herpes Virus and Their Suppression
HENLE, Werner Children's Hospital 5 R01 CA 24779-03	Studies on Epstein-Barr Virus Determined Antigens
HINZE, Harry C University of Wisconsin (Madison) 5 R01 CA 21195-04	Vaccination Against an Oncogenic Herpes Virus
HIRSCH, Martin S Massachusetts General Hospital 5 R01 CA 12464-11	Immune Reactivity and Oncogenic Virus Infections
HORWITZ, Marshall S Yeshiva University 5 R01 CA 11512-12	Adenovirus DNA Synthesis and Polypeptide Assembly
HOWETT, Mary K Pennsylvania State Univ. (Hershey) 5 R01 CA 25305-02	In Vivo Cocarcinogenesis of Chemicals and Viruses
HSU, Ming-Ta Rockefeller University 5 R01 CA 19073-05	Basic Mechanism of Viral Oncogenesis
HUANG, Eng-Shang University of North Carolina (Chapel Hill) 5 R01 CA 21773-03	Cytomegaloviruses and Human Malignancy

HUNTER, Anthony R
Salk Institute for Biological Studies
5 RO1 CA 17096-07

Macromolecular Synthesis and Cell Growth Control

HUNTER, Anthony R
Salk Institute for Biological Studies
5 RO1 CA 28458-02

Viral Transforming Proteins

HYMAN, Richard W
Pennsylvania State Univ. Hershey Med. Ctr.
2 RO1 CA 16498-07

Malignancy and DNA Homology Among the Herpes Viruses

ISOM, Harriet C
Pennsylvania State Univ Hershey Med Ctr
5 RO1 CA 23931-04

Regulation of Differentiation in Hepatocytes In Vitro

KELLEMS, Rodney E
Baylor College of Medicine
5 RO1 CA 24618-03

Control of Host Gene Expression by DNA Tumor Viruses

KIEFF, Elliott D
University of Chicago
5 RO1 CA 17281-07

EBV Interaction with Lymphoblasts In Vitro & In Vivo

KIT, Saul
Baylor College of Medicine
5 RO1 CA 06656-18

Biochemical Aspects of Viral Carcinogenesis

KLEIN, George
Karolinska Institutet
1 RO1 CA 30264-01

Immune Effector Mechanisms in EBV-Carrying Patients

KNIPE, David M
Harvard University
5 RO1 CA 26345-02

Genetics of Herpes Virus Transformation

KOWALSKI, David
Roswell Park Memorial Institute
5 RO1 CA 23996-03

Role of DNA Relaxing Enzyme in SV40 DNA Replication

LANCASTER, Wayne D
Case Western Reserve University
5 R23 CA 24505-03

Bovine Papilloma Virus DNA and Neoplasia

LANCASTER, Wayne D
Case Western Reserve University
1 RO1 CA 28507-01

Papillomavirus DNA and Antigens in Human Neoplasms

LANDERS, Terry A
Univ of Texas Hlth. Sci. Ctr. (Houston)
5 RO1 CA 21350-03

Role of Tumor Virus Structural Proteins in Infection

LEBOWITZ, Jacob University of Alabama (Birmingham) 2 RO1 CA 17077-07	Circular DNA--Implications for Cancer and Drug Resistance
LEHMAN, John M University of Colorado Medical Center 2 RO1 CA 16030-06	Pathology of Neoplastic Transformation
LEVINE, Arnold J State Univ. New York (Stony Brook) 5 RO1 CA 28033-01	DNA Replication in SV40 Infected Cells
LEVINE, Arnold J State Univ. of New York (Stony Brook) 5 RO1 CA 28127-02	SV40 Cellular Antigens and Host Range
LIEF, Florence S University of Pennsylvania 5 RO1 CA 13007-06	Human Wart Virus as an Oncogenic Agent
LIVINGSTON, David M Sidney Farber Cancer Institute 2 RO1 CA 15751-08	Structure and Function of SV40 Non-Virion Proteins
LIVINGSTON, David M Sidney Farber Cancer Institute 5 RO1 CA 24715-03	Isolation and Function of Small SV-40 T Antigen
LUFTIG, Ronald B University of South Carolina 5 RO1 CA 28078-03	Viral Interaction of Microtubules in Cancer Cells
MC DOUGALL, James Fred Hutchinson Cancer Res. Cen. 1 RO1 CA 29350-01	The Biology of Transformation Herpesviruses
MANN, Kristine E University of Alaska 5 RO1 CA 26048-02	SV40 Tumor Antigen in SV40 Nucleoprotein Complexes
MARTIN, Jonathan Tulane University 1 RO1 CA 29631-01	Molecular Analysis of JC Virus Interaction With Cells
MEINKE, William J University of Arizona 5 RO1 CA 23675-03	Detection of Human Wart Viral DNA in Malignant Tissue
MILLER, I George, Jr Yale University 5 RO1 CA 12055-10	Studies of Epstein-Barr Virus

MILLETTE, Robert L Wayne State University 5 RO1 CA 21065-05	Herpes Simplex Virus Gene Regulation
MUNNS, Theodore W Washington University 5 RO1 CA 27801-03	Characterization of RNA/DNA in Oncogenic Systems
NATHANS, Daniel Johns Hopkins University 5 PO1 CA 16519-07	Program on Molecular Biology of Viral Tumorigenesis
NONOYAMA, Meihan Life Sciences Biomed Research Inst. 5 RO1 CA 21665-06	Oncogenicity of Epstein-Barr Virus
NONOYAMA, Meihan Life Sciences Biomed Research Inst. 5 RO1 CA 29353-02	Marek's Disease Virus: Transformation and Oncogenesis
O'NEILL, Frank J University of Utah 2 RO1 CA 15141-07	Control of Nuclear Events in Normal and Neoplastic Cells
OZER, Harvey L Hunter College 2 RO1 CA 23002-05	Host Functions Related to Tumor Virus Infection
PAGANO, Joseph S University of North Carolina 5 PO1 CA 19014-05	DNA Virus Genomes, Oncogenesis and Latency
PASS, Franklin University of Minnesota (Minneapolis) 5 RO1 CA 25462-03	Human Papilloma Virus and Malignant Disease
PEARSON, Gary R Mayo Foundation 5 RO1 CA 20679-05	Biochemistry of Herpes Virus-Induced Membrane Antigens
PEARSON, GARY R Mayo Foundation 5 RO1 CA 24123-03	Humoral Response to Herpes and Epstein-Barr Virus
PEARSON, George D Oregon State University 2 RO1 CA 17699-07	Replication Map of an Oncogenic Virus
POLLACK, Robert E Columbia University 5 RO1 CA 25066-04	Oncogenic Virus Gene Control of Cell Growth and Shape

PRIVES, Carol L Columbia University 5 RO1 CA 26905-02	Function and Expression of SV40 Viral Tumor Antigens
RAPP, Fred Pennsylvania State University 5 PO1 CA 27503-02	Herpesviruses and Neoplasia
RASKA, Karel, Jr. Rutgers Medical School 5 RO1 CA 21196-05	Low Molecular Weight RNA in Adenovirus Infections
RASKAS, Heschel J Washington University 5 RO1 CA 16007-06	Gene Expression of Oncogenic Viruses
RAYMENT, Ivan Brandeis University 5 R23 CA 27260-03	X-Ray Diffraction Studies on Polyoma Virus
REKOSH, David M State Univ. of New York (Buffalo) 5 RO1 CA 25674-03	Adenovirus Early Gene Function and DNA Replication
RICCIARDI, Robert Wistar Institute 1 RO1 CA 29797-01	Organization and Expression of Adenovirus Genes
ROBERTS, Bryan E Harvard University 5 RO1 CA 27447-03	Organization and Expression of Genes in Viral DNAs
ROEDER, Robert G Washington University 5 RO1 CA 16640-07	Regulation of Adenovirus Gene Expression
ROIZMAN, Bernard University of Chicago 5 RO1 CA 08494-17	Mechanisms of Viral Infection in Relation to Cancer
ROMAN, Ann Indiana Univ. (Indianapolis) 1 RO1 CA 29318-01	Regulation of Papovavirus Replication
ROSENTHAL, Ken S Northeastern Ohio Universities 5 R23 CA 28342-02	Herpes Simplex Virus Glycoproteins and Infection
ROTHSCHILD, Henry Louisiana State University 5 RO1 CA 27943-02	Structure and Function of an SV40 Thermoinducible Mutant

RUNDELL, Mary K
Northwestern University
2 RO1 CA 21327-04

Structural Analysis of the SV40 Tumor Antigen

SCHAFFER, Priscilla A
Sidney Farber Cancer Institute
5 RO1 CA 20260-05

Transforming Genes of Herpes Simplex Virus

SCHIERMAN, Louis W
University of Georgia
5 RO1 CA 30109-02

Immunogenetic Study of a Herpes Virus Induced Lymphoma

SCHMIDT-ULLRICH, Rupert
Tufts-New England Medical Ctr.
5 RO1 CA 23642-03

Characterization of SV40-Specific Membrane Antigens

SHAH, Keerti V
Johns Hopkins University
2 RO1 CA 13478-10

Investigation of SV40-Related Infections of Man

SHENK, Thomas E
State Univ. New York (Albany)
5 RO1 CA 28919-02

Structure and Function of DNA Tumor Virus Genomes

SILVERSTEIN, Saul J
Columbia University
5 RO1 CA 17477-06

Molecular Biology of Herpes Virus

SIMMONS, Daniel T
University of Delaware
5 RO1 CA 25942-02

Origin and Function of SV40-Specific TAU Antigens

SPELSBERG, Thomas C
Mayo Foundation
5 RO1 CA 25340-03

A New Class of Epstein-Barr Virus Nuclear Antigen

ST. JEOR, Stephen C
University of Nevada
5 RO1 CA 28089-03

Herpesvirus-Induced Malignancy

STEINBERG, Bettie M
L.I. Jewish-Hillside Med. Ctr.
7 R23 CA 29530-01

Regulation of Reversion in SV40 Transformed Rat Cells

STEINBERG, Mark L
New York University
5 R23 CA 27869-02

Viral Transformation of Human Keratinocytes

STEVENS, Jack G
University of California (LA)
5 RO1 CA 18151-06

Analysis of the Shope Papilloma-Carcinoma System

STROHL, William A Rutgers Medical School 5 RO1 CA 08851-16	Viral Carcinogenesis and Latency
STROMINGER, Jack L Sidney Farber Cancer Institute 5 PO1 CA 21082-05	Molecular Basis of Viral Oncogenesis
STROMINGER, Jack L Sidney Farber Cancer Institute 5 RO1 CA 24926-02	Study of Epstein-Barr Nuclear Antigen
SUMMERS, William C Yale University 2 RO1 CA 13515-09	Genetic Study of Animal Viruses
TAMM, Igor Rockefeller University 5 RO1 CA 18608-21	Virus-Induced Alterations in Animal Cells
TEGTMEYER, Peter J State Univ New York (Stony Brook) 5 RO1 CA 18808-06	Mechanisms of Viral Oncogenesis: SV40 Gene Function
TEGTMEYER, Peter J State Univ. New York (Stony Brook) 5 PO1 CA 28146-02	Tumor Virus-Host Interaction
TENEN, Daniel G Sidney Farber Cancer Institute 5 R23 CA 26018-03	SV40 T Antigen Binding Sites in Cellular DNA
TEVETHIA, Mary J Penn State Univ. Hershey Med. Ctr. 2 RO1 CA 24694-04	Mutagenesis of Specific Regions of the SV40 Genome
TEVETHIA, Satvir S Penn State Univ Hershey Med Ctr 5 RO1 CA 25000-03	Biology of SV40 Specific Transplantation Antigen
THEIS, Gail A New York Medical College 5 RO1 CA 18904-06	Responses of Lymphocytes to an Oncogenic Herpes Virus
THORLEY-LAWSON, David Sidney Farber Cancer Institute 5 RO1 CA 28737-02	Epstein Barr Virus Membrane Antigen
TIBBETTS, Clark J Univ of Connecticut Health Center 5 RO1 CA 17130-06	Adenovirus Genome Expression--Physical Mapping Studies

TJIAN, Robert University of California (Berkeley) 5 RO1 CA 25417-02	The SV40 Tumor Antigen
TOPP, William C Cold Spring Harbor Laboratory 5 RO1 CA 24830-03	SV40 Early Gene Products and Viral Oncogenesis
TROY, Frederic A University of California (Davis) 5 RO1 CA 17327-06	Membrane Bound Enzymes, Tumor Antigens and Malignancy
ULTMANN, John E University of Chicago 5 PO1 CA 19264-05	Viral Oncology Research Center Program
VARSHAVSKY, Alexander Mass. Institute of Technology 1 RO1 CA 30376-01	Studies on the SV40 Virus Structure and Replication
VELICER, Leland F Michigan State University 5 RO1 CA 23408-03	Marek's Disease Herpes Virus Antigens
VILLARREAL, Luis P Univ. of Colorado Medical Center 5 RO1 CA 25819-02	Gene Expression of a Small DNA Tumor Virus: SV40
WAGNER, Edward K University of California (Irvine) 5 RO1 CA 11861-11	Viral RNA Synthesis Control in Herpes Virus Infection
WATSON, James D Cold Spring Harbor Laboratory 5 PO1 CA 13106-10	Cold Spring Harbor Laboratory Cancer Research Center
WEISSMAN, Sherman M Yale University 5 PO1 CA 16038-07	Program on the Molecular Basis of Viral Transformation
WEISSMAN, Sherman M Yale University 5 RO1 CA 16106-06	Function of SV40 Gene Products
WILLIAMS, James F Carnegie-Mellon University 5 RO1 CA 21375-04	Genetic Analysis of Adenoviruses
WILSON, Geoffrey Yale University 1 R23 CA 29183-01	Production of SV40-Specified Antigens in E Coli

WILSON, John H
Baylor College of Medicine
5 R01 CA 15743-09

Oncogenic Mechanisms: SV40 and Host Genetic Analysis

WOLD, William S
St. Louis University
5 R23 CA 24710-03

Adenovirus 2 Coded Early Glycoprotein

WU, Guang-Jer
Emory University
5 R01 CA 25270-03

Regulation of Transcription

BIOLOGICAL CARCINOGENESIS BRANCH

GRANTS ACTIVE DURING FY 81

RNA VIRUS STUDIES (I)

Investigator/Institution/Grant Number	Title
ARLINGHAUS, Ralph B. Univ. of Texas System Cancer Ctr. 5 RO1 CA 25465-02	Biosynthesis and Characterization of Murine Oncornavirus
AUGUST, J. Thomas Johns Hopkins University 5 RO1 CA 19471-05	Genetic Expression of Cellular Oncornaviruses
BACHELER, Lee T. Salk Institute for Biological Studies 5 RO1 CA 22829-03	Organization of Integrated Tumor Virus Genomes
BACHELER, Lee T. Temple University 2 RO1 CA 29519-02	Organization and Expression of Leukemia Virus Genomes
BALAZS, Ivan Sloan Kettering Inst. for Can. Res. 5 RO1 CA 24008-02	RNA Tumor Virus Gene Expression During the Cell Cycle
BALTIMORE, David Massachusetts Institute of Technology 5 PO1 CA 26717-02	Molecular Analysis of Oncogenic Viruses
CARDIFF, Robert D. Univ. of California (Davis) 5 RO1 CA 21454-05	MTV Gene Amplification and Expression
CERNY, Jan Harvard University 5 RO1 CA 14922-08	Regulatory Mechanisms of Neoplasia
COGGIN, Joseph H., Jr. University of South Alabama 2 RO1 CA 23491-04	Etiology of a Lymphoma Epizootic in Hamsters
COHEN, J. Craig Tulane University School of Medicine 1 RO1 CA 30358-01	Role of Endogenous Viruses in Mammary Carcinogenesis
COMPANS, Richard W. University of Alabama (Birmingham) 5 RO1 CA 18611-07	Molecular Studies of Oncorna and Arenaviruses

DARNELL, James E., Jr. Rockefeller University 5 PO1 CA 18213-06	Correlated Program in Viral Oncology
DATTA, Syamal K. New England Med. Ctr. Hosp. 5 RO1 CA 19575-06	Murine Leukemia Viruses in New Zealand Mice
DAVIDSON, Norman R. California Institute of Technology 5 RO1 CA 25991-02	Sequence Organization of Integrated Tumor Virus Genomes
DINA, Dino Yeshiva University 5 RO1 CA 24223-03	Regulation of Murine RNA Tumor Virus Gene Expression
DURAN-REYNALS, Maria L. Yeshiva University 5 RO1 CA 07160-16	Possible Neoplastic Effects of Non-Neoplastic Viruses
EAST, James L. Univ. of Texas System Cancer Ctr. 3 RO1 CA 16781-06S1	Relatedness of RNA Tumor Viruses and Human Neoplasia
ECKNER, Robert J. Boston University 5 RO1 CA 19562-06	Biological and Physical Properties of Friend Virus
ELDER, John H. Scripps Memorial Hospital 5 RO1 CA 25533-03	Sequence Studies of the gp70's of Recombinant Retrovirus
ESSEX, Myron E. Harvard University 5 RO1 CA 13885-07	Oncornavirus-Associated Cell Membrane Antigens
ESSEX, Myron E. Harvard University 2 RO1 CA 18216-05	Study of (FLV) Leukemia Virus
FAMULARI, Nancy G. Sloan-Kettering Mem. Inst. for Cancer Res. 5 RO1 CA 27950-02	Influence of MuLV env and gag genes in leukemogenesis
FAN, Hung Y. Salk Institute for Biological Studies 5 RO1 CA 15747-08	Expression and Localization of C-Type Virus Genes
FAN, Hung Y. Salk Institute for Biological Studies 5 RO1 CA 28617-02	Studies of Murine Leukemia Virus Integration

FELLER, William F.
Georgetown University
5 R01 CA 17623-04

Characterization of DNA Polymerases in Human Milk

FERRER, Jorge F.
University of Pennsylvania
5 P01 CA 14193-08

Comparative Leukemia Studies Unit

FERRER, Jorge F.
University of Pennsylvania
3 P01 CA 14193-08S1

Comparative Leukemia Studies Unit

FLEISSNER, Erwin J.
Sloan-Kettering Inst. for Cancer Res.
5 R01 CA 15297-08

Viral and Mouse Genes in Leukemia Virus Infection

FLEISSNER, Erwin J.
Sloan-Kettering Inst. for Cancer Res.
5 P01 CA 16599-07

Oncogenic Viruses Program Project

FRIEND, Charlotte
Mount Sinai School of Medicine
5 R01 CA 10000-16

Filterable Agents and Tumor Induction in Mice

GERARD, Gary F.
St. Louis University
5 R01 CA 20335-03

Mammalian RNA Tumor Virus Reverse Transcription

GIRARDI, Anthony J.
Institute for Medical Research
5 R01 CA 24940-03

Immunologic Studies in Mouse and Human Breast Cancer

GOFF, Stephen P.
Columbia University
1 R01 CA 30488-01

Construction and Analysis of Retrovirus Mutants

GRAVES, Donald C.
Univ. of Oklahoma Health Science Ctr.
5 R23 CA 23980-03

Studies on the Bovine Leukemia Virus

GREENBERGER, Joel S.
Sidney Farber Cancer Institute
5 R01 CA 26785-02

Requirements for Spontaneous Leukemogenesis In Vitro

HAAS, Martin
Salk Institute for Biological Studies
5 R01 CA 30146-01

Viral Malignant Lymphomagenesis in X-Irradiated mice

HANKINS, William D.
Vanderbilt University
5 R01 CA 26306-03

A Friend Virus Transformation System In Vitro

HASELTINE, William A. Sidney Farber Cancer Institute 1 R01 CA 29294-01	Molecular Biology of Leukemia and Sarcoma Retroviruses
HAYS, Esther F. Univ. of California (Los Angeles) 5 R01 CA 12386-09	Development of Lymphoma in the Thymus
HINES, David L. Michigan Cancer Foundation 5 R01 CA 25234-03	Differentiation of Abelson Virus Leukemic Cells
HOOVER, Edward A. Ohio State University 2 R01 CA 22527-05	Pathogenesis of Animal Leukemia
HOPKINS, Nancy H. Massachusetts Institute of Technology 5 R01 CA 19308-06	Endogenous Viruses of BALB/c Mice
HUNTER, Eric University of Alabama (Birmingham) 5 R01 CA 27834-02	Genetics of Primate "D" Type Retroviruses
JONES, Joe M. Univ. of Arkansas Med. Sciences Camp 5 R01 CA 23687-03	Genetic Control of Neoplasms in Rats
KABAT, David Univ. of Oregon Hlth. Sciences Ctr. 5 R01 CA 23032-11	Control of Gene Expression in in Differentiating Cells
KABAT, David Univ. of Oregon Hlth Sciences Ctr. 5 R01 CA 25810-03	Leukemogenesis by Friend Spleen Focus Forming Virus
KAPLAN, Henry S. Stanford University 5 R01 CA 03352-25	Biological Aspects of Carcinogenesis by Radiation
KAPLAN, Henry S. Stanford University 5 R01 CA 25619-03	C57BL Mouse Retroviruses: Molecular Characterization
KAPLAN, Henry S. Stanford University 1 R01 CA 29079-01	Studies of Retroviruses from Human Lymphoma Cells
KEMP, Robert G. Univ. of Hlth. Sci./Chicago Med. Sch. 5 R01 CA 21631-05	Metabolic Events in Virus-Induced Leukemogenesis

KINGSBURY, David T.
University of California (Irvine)
5 RO1 CA 24748-03

Cloning and Analysis of Murine Endogenous Virogenes

LASFARGUES, Etienne Y.
Institute for Medical Research
5 RO1 CA 08515-15

Tissue Culture Studies of the Mammary Tumor Virus

LERNER, Richard A.
Scripps Clinic and Research Fdn.
5 RO1 CA 25325-03

Immunologic Probing of Sarcoma Diversity

LERNER, Richard A.
Scripps Clinic and Research Fdn.
5 PO1 CA 27489-02

Immunological and Pathological Consequences of Endogenous Retroviral Expression

LEVY, Jay A.
Univ. of California (San Francisco)
3 RO1 CA 13086-09S1

C-Type Viruses in Autoimmune Disease and Neoplasia

LILLY, Frank
Yeshiva University
5 RO1 CA 19873-05

Genetic Control of Resistance to Friend Virus Disease

LILLY, Frank
Yeshiva University
5 RO1 CA 19931-05

Mechanism of the H-2 Effect on Viral Leukemogenesis

LILLY, Frank
Yeshiva University
5 RO1 CA 26010-02

Genetic Resistance to Spontaneous Leukemia in Mice

LO BUE, Joseph
New York University
5 RO1 CA 12815-08

Pathophysiology of a Virally Induced Murine Leukemia

LUFTIG, Ronald B.
University of South Carolina
5 RO1 CA 28077-03

Assembly of Murine Leukemia Viruses

MANLY, Kenneth F.
Roswell Park Memorial Institute
5 RO1 CA 21217-03

Molecular Pathogenesis of Virus-Induced Leukemia

MARCUS, Stuart L.
Sloan-Kettering Inst. for Can. Res.
5 RO1 CA 18369-06

RLV DNA Polymerase Enzymology and Radioimmunoassay

MARIANI, Toni N.
University of Minnesota (Minneapolis)
3 RO1 CA 12929-09S1

Interrelationship Between Malignancy and Immunity

MAYER, Allen J. New York University 5 R01 CA 23834-03	Endogenous Virus Expression and Leukemogenesis
MC DONALD, Thomas L. University of Nebraska 5 R01 CA 28205-03	Virus-Induced Blocking Factors and Tumor Enhancement
McGRATH, Charles M. Michigan Cancer Foundation 5 R01 CA 18175-06	Endogenous Virus and Hormones in Mammary Cancer
MELLORS, Robert C. Hospital for Special Surgery 5 R01 CA 14928-16	Type C RNA Virus and Immunopathology
MERUELO, Daniel New York University 5 R01 CA 22247-05	Genetic Control of Virus-Induced Leukemia
MODAK, Mukund J. Sloan-Kettering Institute 5 R01 CA 21404-05	Molecular Effects of Enzymatic DNA Synthesis
NICOLSON, Margery O. Children's Hospital of Los Angeles 5 R01 CA 26199-03	Integrated Infective Type-C Retroviral Genomes
NOWINSKI, Robert C. Fred Hutchinson Cancer Research Center 5 R01 CA 18074-06	Immunological Aspects of Mouse Leukemias
NOWINSKI, Robert C. Fred Hutchinson Cancer Research Center 5 R01 CA 24706-03	Mouse Leukemia Viruses: Immunogenetic Studies
OLSEN, Richard G. Ohio State University 1 R01 CA 30338-01	FeLV Leukemogenesis and Preneoplastic Lesions
OZANNE, Bradford W. Univ. of Texas Hlth. Sci. Ctr. (Dallas) 5 R01 CA 23043-03	Peptide Transforming Factors from Transformed Cells
PARKS, Wade University of Miami 5 R01 CA 27890-02	MMTV in Spleen Lymphoid Cells: Immunologic Effect
PIKÓ Lajos Veterans Administration Hospital 5 R01 CA 24989-03	Gene Expression in Early Mouse Development

PINCUS, Theodore P. Wistar Inst. of Anatomy and Biology 5 R01 CA 24744-03	Natural Host Control Mechanisms in Leukemia
PINCUS, Theodore P. Wistar Inst. of Anatomy and Biology 7 R01 CA 30145-01	Genetic Restriction of Endogenous Retroviruses
PROFFITT, Max R. Cleveland Clinic Hospital 2 R01 CA 20242-06	Autoimmunity and Virus-Induced Leukemia
RASCHKE, William C. La Jolla Cancer Research Foundation 7 R01 CA 30903-01	Characterization of the Abelson Leukemia Virus
RASHEED, Suraiya University of Southern California 5 R01 CA 27246-02	Leukemia and Sarcoma Genes in Cellular Transformation
RICH, Marvin A. Michigan Cancer Foundation 3 R01 CA 14100-08S1	The Viral Etiology of Cancer
ROSENBERG, Naomi E. Tufts University 5 R01 CA 24220-03	Abelson Leukemia Virus Transformation of Lymphocytes
ROY-BURMAN, Pradip University of Southern California 5 R01 CA 26809-02	Oncodevelopmental Gene Expression in Feline Leukemia
SARKAR, Nurul H. Sloan-Kettering Inst. for Can. Res. 2 R01 CA 17129-07	Components of the Mouse Mammary Tumor Virus
SCHWARTZ, Robert S. Tufts University 5 P01 CA 24530-03	Experimental Leukemogenesis
SNYDER, Harry W., Jr. Sloan-Kettering Inst. for Can. Res. 5 R23 CA 24357-03	Chemical Nature of Leukemia Antigens
SOMERS, Kenneth D. Eastern Virginia Medical School 5 R01 CA 28474-02	Cellular Transformation by MSV
SPIEGELMAN, Sol Columbia University 5 P01 CA 23767-03	Molecular Virology

STAAL, Stephen P. Johns Hopkins University 5 R01 CA 26089-03	Studies on Leukemogenesis in AKR Mice
STEFFEN, David L. Worcester Fdn. for Experimental Biology 1 R01 CA 30674-01	Mechanisms of Viral and Nonviral Leukemogenesis
THACH, Robert E. Washington University 5 R01 CA 13008-10	Replication of Virulent and Oncogenic Viruses
TOMANA, Milan University of Alabama (Birmingham) 5 R01 CA 19918-06	Biochemistry of Carbohydrates in Cell Surface Components
TSUCHIDA, Nobuo Wistar Inst. of Anatomy and Biology 5 R01 CA 22701-04	Host/Virus Interaction in Ki-MSV Genome Expression
TUCKER, Henry S. Virginia Commonwealth University 5 R23 CA 24172-03	Macrophage Role in Immunity to Murine Leukemia Virus
VAIDYA, Akhail Hahneman Medical College and Hospital of Philadelphia 5 R01 CA 22413-05	Etiological Studies of Mammary Carcinoma
VARMUS, Harold E. Univ. of California (San Francisco) 2 R01 CA 19287-06	Molecular Biology of Mouse Mammary Tumor Virus
VELICER, Leland F. Michigan State University 5 R01 CA 24937-03	Expression of Genes for Tumor-Specific Antigens
VERMA, Inder M. Salk Institute for Biological Studies 5 R01 CA 16561-07	Reverse Transcriptase from RNA Tumor Viruses
VERMA, Inder M. Salk Institute for Biological Studies 2 R01 CA 21408-05	Genetic Organization of RNA Tumor Viruses
VOGT, Marguerite M. Salk Institute for Biological Studies 2 R01 CA 13608-09	Viral Gene Functions Involved in Transformation
WATSON, James D. Cold Spring Harbor Laboratory 5 R13 CA 02809-26	Support for Symposia on Quantitative Biology

WATSON, James D. Cold Spring Harbor Laboratory 5 R13 CA 16224-08	Support for Cancer Research Center Courses
WEIGAND, Ray A. Michigan Cancer Foundation 5 R01 CA 23470-04	Assembly and Maturation of Murine Leukemia Viruses
WEINBERG, Robert A. Massachusetts Institute of Technology 5 R01 CA 17537-07	Interaction of Sarcoma and Leukemia Genomes
WHEELOCK, E. Frederick Thomas Jefferson University 5 R01 CA 24115-03	Role of Endogenous Viruses in Tumor Dormant States
WITTE, Owen N. University of California 5 R01 CA 27507-02	Transformation by Abelson Murine Leukemia Virus
WONG, Paul K. Univ. of Illinois (Urbana-Champaign) 5 R01 CA 17695-06	Genetic Studies of Murine Leukemia Viruses
YAMAMOTO, Keith Univ. of California (San Francisco) 5 R01 CA 20535-05	Mechanisms of Gene Regulation by Steroid Receptor Proteins
YANG, Wen K. Oak Ridge National Laboratory 1 R01 CA 30308-01	Mechanism of Fv-1 Restriction of Murine Leukemia Viruses
YOHN, David Ohio State University 1 R13 CA 30226-01	Annual Meeting of Leukemia Society of America
YOSHIMURA, Fayth K. Fred Hutchinson Cancer Research Center 5 R01 CA 25461-03	DNA Forms of Murine Leukemia Virus

GRANTS ACTIVE DURING FY 81

RNA VIRUS STUDIES (II)

Investigator/Institution/Grant Number	Title
ABELSON, John N. U. California (San Diego) 5 RO1 CA 10984-13	Structure and Function of Ribonucleic Acids
ASTRIN, Susan M. Inst. for Cancer Research, Philadelphia 5 RO1 CA 27797-02	Control of Expression of Endogenous Viral Genes
AXEL, Richard Columbia University 5 RO1 CA 16346-07	Molecular Control of Chromatin Transcription
BALDUZZI, Piero C. University of Rochester 5 RO1 CA 15716-07	Genetic Studies of RNA Tumor Viruses
BALUDA, Marcel A. Univ. of California (Los Angeles) 5 RO1 CA 10197-15	Tumor Induction by Avian Myeloblastosis Virus
BEEMON, Karen L. Salk Inst. for Biological Studies 5 RO1 CA 23896-03	Expression of RNA Tumor Virus Genes
BISHOP, John M. Univ. of California (San Francisco) 5 RO1 CA 12705-11	Rous Sarcoma Virus: Replication and Cell Transformation
BOETTINGER, David E. University of Pennsylvania 5 RO1 CA 16502-07	Genetic Analysis of RNA Tumor Viruses
BOETTINGER, David E. University of Pennsylvania 1 RO1 CA 30383-01	Virus Induced Myeloid Leukemia
BOSE, Henry B. University of Texas 5 RO1 CA 27003-02	Tranforming and Helper Virus Sequences in REV-Transformed Cells
BREWER, John I. Northwestern Univ. 1 RO1 CA 29461-01	Trophoblastic Tumors: New Organism/Immunology/Therapy
BRILES, Worthie E. Northern Illinois University 5 RO1 CA 12796-10	Immunogenetics of Tumor Related Alloantigens

BRUGGE, Joan S. SUNY--at Stony Brook 5 R01 CA 27951-02	The ASV Transforming Protein and its Cellular Homologue
CARBON, John A. U. California (Santa Barbara) 5 R01 CA 11034-13	Studies on Gene Organization and Expression
CASPAR, Donald L. Brandeis University 5 R01 CA 15468-08	Assembly of Viruses, Membranes and Tissues
CHIRIKJIAN, Jack G. Georgetown University 5 R01 CA 16914-06	RNA Modifying Enzymes in Normal and Neoplastic Cells
COFFIN, John M. Tufts University 5 R01 CA 17659-06	Relationship of Avian Tumor Virus RNA and Host Genome
COFFIN, John M. Tufts University 5 R01 CA 27108-02	Mechanism of Variability of Tumor Virus RNA
COLLINS, Carolyn J. University of Virginia 5 R01 CA 24137-03	Integration of RNA Tumor Viruses in Mammalian Cells
COOPER, Geoffrey M. Sidney Farber Cancer Inst 5 R01 CA 18689-06	Infectious DNA for Endogenous RNA Tumor Virus Genes
CRITTENDEN, Lymen B. U.S. Dept. of Agriculture 1 R01 CA 29656-01	Avian Endogenous Retroviral Gene Expression
DAHLBERG, James E. University of Wisconsin (Madison) 5 R01 CA 15166-08	Small RNAs of RNA Tumor Viruses
DEL VILLANO, Bert C. Cleveland Clinic Hospital 5 R01 CA 25789-03	Biological Studies of Epididymal Glycoprotein, gp70
DODGE, William H. Wake Forest University 5 R01 CA 19275-06	Normal and Leukemic Granulopoiesis in the Chicken
DUESBERG, Peter H. University of California (Berkeley) 5 R01 CA 11426-13	Molecular and Genetic Analyses of RNA Tumor Viruses

EHRLICH, Melanie Tulane Medical School 2 RO1 CA 19942-05	5-Methylcytosine in DNA: Cancer and Development
EISENMAN, Robert N. Fred Hutchinson Cancer Research Cen. 5 RO1 CA 20525-04	Control Mechanisms in Avian Oncornavirus Replication
ERIKSON, Raymond L. University of Colorado Medical Cen. 5 RO1 CA 21117-16	Biosynthesis of Viral RNA
ERIKSON, Raymond L. University of Colorado Medical Cen. 5 RO1 CA 21326-03	Avian Sarcoma Virus Transformation-Specific Antigens
FARAS, Anthony J. University of Minnesota (Minneapolis) 5 RO1 CA 18303-07	RNA-Directed DNA Polymerase and 70S RNA of Oncornavirus
FARAS, Anthony J. University of Minnesota (Minneapolis) 5 RO1 CA 20011-05	Studies of the Mechanism of Retrovirus Proviral DNA Synthesis
FARAS, Anthony J. University of Minnesota (Minneapolis) 5 RO1 CA 26387-03	Reversion of Rous Sarcoma Virus-Transformed Cells
GANESAN, Adayapalam Stanford University 5 RO1 CA 16896-21	Genetics of Bacteria
GOLDBERG, Allan R. Rockefeller University 5 RO1 CA 13362-10	RSV Functions Involved in Transformation
GRANDGENETT, Duane P. St. Louis University 5 RO1 CA 16312-08	Avian Retrovirus DNA Synthesis and its Regulation
GRAY, Horace B. University of Houston 5 RO1 CA 11761-11	Hydrodynamics of Circular DNA Forms
HANAFUSA, Hidesaburo Rockefeller University 2 RO1 CA 14935-09	Cellular Alteration Induced by Rous Sarcoma Virus
HARRISON, Stephen C. Harvard University 5 RO1 CA 13202-10	Virus Structure and Assembly

HAYWARD, William S. Rockefeller University 2 RO1 CA 16668-08	RNA Tumor Virus Gene Expression
HOLTZER, Howard University of Pennsylvania 5 RO1 CA 18194-07	Conversion of Embryonic Cells into Transformed Cells
HUMPHRIES, Eric H. Univ. of Texas Hlth Sci Ctr (Dallas) 5 RO1 CA 23041-03	Expression of Avian Sarcoma Virus in Rat Fibroblasts
KOPROWSKI, Hilary Wistar Institute 5 PO1 CA 21124-05	Genetics and Virology of Cancer
KRZYZEK, Richard A. University of Minnesota (Minneapolis) 5 R23 CA 23833-03	Control of Avian Oncornavirus Genes in Mammalian Cells
KUNG, Hsing-Jien Michigan State Universit 5 RO1 CA 24798-03	Recombination and Replication of Avian Sarcoma Viruses
LAI, Michael M. University of Southern California 2 RO1 CA 16113-07	Structure and Replication of RNA Tumor Viruses
LEIS, Jonathan P. Case Western Reserve University 5 RO1 CA 27414-03	Processing and Translation of Tumor Virus RNA In Vitro
LINIAL, Maxine L. Fred Hutchinson Cancer Research Ctr. 5 RO1 CA 18282-06	Viral Coded Functions in Rous Sarcoma Virus
MAGUN, Bruce E. University of Arizona 5 RO1 CA 20913-05	Growth Regulatory Mechanisms in Viral Transformation
MARCUS, Philip I. University of Connecticut 5 PO1 CA 14733-08	Study of Genetics
MARTIN, G. Steven University of California (Berkeley) 5 RO1 CA 25464-03	Transformation of Differentiating Cells
MARTIN, G. Steven University of California (Berkeley) 5 RO1 CA 17542-06	Genetics of RNA Tumor Viruses
MASON, William S. Institute for Cancer Research 5 RO1 CA 26012-02	Replication of Rous Sarcoma Virus

MILMAN, Gregory Johns Hopkins University 5 R01 CA 21650-06	Biochemistry of Mutation in Human Cells
MOLDOW, Charles F. University of Minnesota (Minneapolis) 3 R01 CA 13722-06S1	Cell Surface Receptors for Oncogenic Viruses
MOSCOVICI, Carlo University of Florida 5 R01 CA 10697-15	Specificity of Avian Myeloblastosis Virus
NADAL-GINARD, Bernardo Yeshiva University 5 R01 CA 26860-02	Transforming Gene of Rous Sarcoma Virus
NEIMAN, Paul E. Fred Hutchinson Cancer Research Center 5 R01 CA 20068-06	Molecular Mechanisms in Neoplasia
NEIMAN, Paul E. Fred Hutchinson Cancer Research Center 5 P01 CA 28151-02	Program in Retroviruses and Cancer
PARSONS, J. Thomas University of Virginia 5 R01 CA 27578-03	Mechanisms of RNA Tumor Virus DNA Integration
PENHOET, Edward E. U. California (Berkeley) 5 R01 CA 20357-03	Control of RNA Synthesis in Eukaryotic Cells
PERDUE, Michael L. University of Kentucky 5 R01 CA 26170-03	Processing of Avian Oncornavirus Messenger RNA
POGO, Beatriz G. Mt. Sinai School of Medicine 2 R01 CA 15953-04	Replication and Expression of Poxviruses
QUIGLEY, James P. Downstate Medical Center 2 R01 CA 16740-06	Proteases in Growth Control and Malignant Transformation
RHODE, Solon L., III Institute of Medical Research of Bennington 7 R01 CA 26801-03A1	Replicon Control in Normal and Transforming Cells
ROBBINS, Phillips W. Mass. Institute of Technology 5 R01 CA 14142-19	Cell and Virus Glycoproteins-Synthesis and Function

ROBINSON, Harriet L.
Worcester Fdn. for Exper. Biology
5 R01 CA 23086-05

Inheritance and Expression of Avian C-Type Viruses

ROBINSON, Harriet L.
Worcester Fdn. for Experimental Biology
5 R01 CA 27223-02

Avian Leukosis Viruses and Cancer

ROHRSCHNEIDER, Larry R.
Fred Hutchinson Cancer Research Center
5 R01 CA 20551-05

Mechanisms of Oncornavirus-Induced Transformation

ROMANO, Antonio H.
Univ. of Connecticut
5 R01 CA 14274-06

Sugar Transport and Malignant Transformation

RUECKERT, Roland R.
University of Wisconsin (Madison)
5 R01 CA 08662-15

Structure and Synthesis of Avian RNA Tumor Viruses

SCHLESINGER, Milton J.
Washington University
5 R01 CA 14311-09

RNA-Envelope Virus Formation in Animal Cells

SCOTT, June R.
Emory University
5 R01 CA 11673-12

Lysogeny and Bacteriophage P1

SEFTON, Bartholomew
Salk Institute
5 R01 CA 17289-06

Viral Membranes: Structure and Biosynthesis

SHANK, Peter R.
Brown University
5 R01 CA 24829-03

Structure and Integration of Avian Sarcoma Virus DNA

SIMPKINS, Henry
University of California (Irvine)
5 R01 CA 25079-02

The Role of Chromatin Structure in Erythroleukemia

SMITH, Ralph E.
Duke University
5 R01 CA 12323-10

Biochemistry of RNA Tumor Virus Replication

STAVNEZER, Edward
Sloan-Kettering Inst. for Can. Res.
5 R01 CA 24163-03

Synthesis and Processing of Avian Retrovirus RNAs

STOLTZFUS, Conrad M.
University of Iowa
5 R01 CA 28051-03

Subunit Structure and Function of Oncornavirus RNA

TAYLOR, John M. Institute for Cancer Research 5 RO1 CA 22651-04	The In Vivo Transcription of RNA Tumor Virus Genomes
TEMIN, Howard M. University of Wisconsin (Madison) 5 PO1 CA 22443-04	Molecular Biology and Genetics of Tumor Viruses
TEREBA, Allan M. St. Jude Children's Research Hospital 5 RO1 CA 28221-02	Localization and Mechanism of Retrovirus Integration
VANAMAN, Thomas C. Duke University Med. Center 1 PO1 CA 30246-01	Regulatory Functions of Protein-Nucleic Acid Interactions
VIOLA, Michael V. U. of Connecticut 5 RO1 CA 27792-02	Pathogenesis of Paget's Disease of Bone
VOGT, Peter K. University of Southern California 5 RO1 CA 13213-09	Interactions Between Avian Tumor Viruses and Their Hosts
VOGT, Peter K. University of Southern California 1 RO1 CA 29777-01	Avian Oncoviruses: Transforming Genes and Proteins
VOGT, Volker M. Cornell University 5 RO1 CA 20081-05	Structure and Assembly of Avian Oncornaviruses
WARNER, Robert C. U. California (Irvine) 5 RO1 CA 12627-11	Studies of Polynucleotides and Nucleic Acids
WATSON, Kenneth F. Univ. of Montana 2 RO1 CA 19729-06	Mechanism of Viral RNA-Directed DNA Polymerization
WEBER, Michael J. U. Illinois (Urbana) 5 RO1 CA 12467-10	Early Cell-Surface Changes in Viral Oncogenesis
WEBER, Michael J. Univ. of Illinois (Urbana-Champaign) 5 RO1 CA 20410-03	Membrane Lipids in Rous Sarcoma Virus Infection
WEINTRAUB, Harold M. Fred Hutchinson Cancer Research Center 5 RO1 CA 26663-02	Cell Transformation by RSV

WEISS, Gary B.
University of Texas
1 R01 CA 31800-01

Infidelity of Human RNA-Directed DNA POlymerase

WEITH, H. Lee
Purdue University
5 R01 CA 23332-03

Sequencing of RSV DNA Integration Sites

WELLS, Robert D.
U. of Wisconsin (Madison)
5 R01 CA 20279-04

DNA Structure and Gene Regulation

BIOLOGICAL CARCINOGENESIS BRANCH

CANCER RESEARCH EMPHASIS GRANTS

Active During FY81

Investigator/Institution/Grant Number	Title
CABRAL, Guy A Virginia Commonwealth University 5 R01 CA 25737-03	Herpesvirus Antigens in Transformed Cells
COURTNEY, Richard J University of Tennessee 5 R01 CA 24564-05	Studies of Purified Herpes Simplex Virus Glycoproteins
FARAS, Anthony J University of Minnesota 5 R01 CA 20011-05	Mechanism of Retrovirus Proviral DNA Synthesis
GREEN, Maurice Saint Louis University Sch. of Med. 5 R01 CA 19996-05	Replication of RNA Tumor Viruses
GREEN, Maurice Saint Louis University Sch. of Med. 5 R01 CA 21824-05	Transforming Proteins of Three Human Adenovirus Groups
GURNEY, Elizabeth T University of Utah 5 R01 CA 21797-05	Growth Control and Viral Gene Expression
HASELTINE, William Sidney Farber Cancer Institute 5 R01 CA 19341-05	Replication of RNA Tumor Viruses
KASAMATSU, Harumi University of California (LA) 5 R01 CA 21768-05	Polypeptides and the Protein-DNA Complex in SV40 Virion
SPEAR, Patricia G University of Chicago 5 R01 CA 21776-05	Herpesvirus Gene Expression in Transformed Cells
VOGT, Peter University of Southern California 5 R01 CA 19725-05	Genetics of RNA Tumor Viruses

WATSON, Kenneth F
University of Montana
5 R01 CA 19729-05

Mechanism of Viral RNA-Directed DNA Polymerization

WONG, Paul K Y
University of Illinois (Urbana)
5 R01 CA 19723-05S1

Genetics Studies of Helper-Independent Mouse Sarcoma Virus

SUMMARY REPORT
CHEMICAL AND PHYSICAL CARCINOGENESIS BRANCH

The Chemical and Physical Carcinogenesis Branch (1) plans, coordinates, and administers a national extramural program of basic and applied research consisting of grants and contracts, collectively concerned with the occurrence and the inhibition of cancer, caused or promoted by chemical or physical agents acting separately or together or in combinations with biological agents; (2) plans, organizes, and conducts meetings of scientists and otherwise maintains contacts with scientists-at-large, to identify and evaluate new and emergent research in and related to the fields of chemical and physical carcinogenesis; (3) provides a broad spectrum of information, advice, and consultation to scientists and to institutional science management officials, relative to NIH and NCI funding and scientific review policies and procedures, preparation of grant applications, and choice of funding instrument, based on individual need; (4) plans, develops, maintains, and allocates research resources necessary for the support of carcinogenesis research of high programmatic interest; and (5) provides NCI management with recommendations concerning funding needs, priorities, and strategies relative to the support of chemical and physical carcinogenesis research, consistent with the current state of development of individual research elements and the promise of potential, new initiatives.

Research grants and contracts assigned to the Chemical and Physical Carcinogenesis Branch are collectively concerned with the achievement of a more effective prevention of human cancer, caused or promoted by chemical or physical agents acting separately or together, or in combinations with biological agents. Research supported under this program bears upon a broad range of subject-matter areas, with principal emphasis on environmental carcinogenesis, mechanisms of action of chemical and physical carcinogens, DNA damage and repair in carcinogenesis, role of endocrine factors in carcinogenesis, experimental inhibition of carcinogenesis, the phenomenon of promotion in carcinogenesis, interspecies comparisons in the response to carcinogen exposure, and the develoment of culture systems for use in carcinogenesis research. The program also supports the development and distribution of a considerable spectrum of research resources relevant to studies on chemical carcinogenesis.

Grants and contracts assigned to this Branch support five complementary categories of carcinogenesis research and related activities: Molecular Carcinogenesis, Carcinogenesis Mechanisms, Chemoprevention, Special Projects, and Research Resources. Molecular Carcinogenesis focuses on changes in physiological compounds and processes produced by carcinogens, effects of carcinogens on cell structure, ultrastructure and function, DNA damage and repair in carcinogenesis, molecular structure-carcinogenicity relationships, and enzymes characteristically associated with the carcinogenesis process. The Carcinogenesis Mechanisms category relates to the absorbtion and body distribution of carcinogens, specificity of transformation, metabolism, activation and inactivation of carcinogens, identification of proximate and ultimate carcinogenic forms, factors which alter the action of carcinogens, and carcinogen-mutagen relationships. Chemoprevention focuses on the inhibition of carcinogenesis by chemical agents. Efforts are devoted to the identification, development, and testing of potential chemopreventive agents, in both in vitro and in vivo systems. Areas of interest include mechanisms of action of chemopreventive agents, binding proteins and receptors, and structure-function relationships. Program Projects and analogous multi- and interdisciplinary contracts are assigned to the Special Projects category. The latter includes studies which seek to define

the role of promotion in environmental carcinogenesis, mechanisms of action of promoters, the role of endocrine factors in carcinogenesis, metabolic patterns in pre-clinical and in early neoplasia, interspecies comparisons in the response to carcinogen exposure, the development of cell culture systems for use in carcinogenesis research, and the sponsorship of carcinogenesis workshops, conferences, and symposia. The Research Resources category, supported solely by contract, is concerned with the synthesis and distribution of selected carcinogens and certain of their metabolites, with particular reference to polynuclear hydrocarbon carcinogens, their metabolic intermediates, and analogous heterosubstituted compounds, as well as the synthesis and distribution of retinoids, including radiolabeled forms.

In terms of increased programmatic emphasis, a Request for Applications (RFA) was announced in the area of Interspecies Comparisons in Carcinogenesis. This RFA seeks to encourage both basic and applied studies which might provide insights and approaches to an understanding of similarities and differences in the response to chemical carcinogens, between experimental animals and humans. The intended emphasis is on: (a) the use of accessible human cells, tissues, body fluids, and excreta, and (b) studies which focus on quantitative relationships with respect to the carcinogenesis process. Analogous new initiatives include the preparation of an RFA in the area of Mechanisms of Biological and Chemical Prevention of Carcinogenesis and the planning of a possible RFA concerned with the role of tumor promoters, hormones, and other cofactors in human cancer causation.

TABLE I

CHEMICAL AND PHYSICAL CARCINOGENESIS BRANCH

	FY 1981			
	CONTRACTS		GRANTS	
	No. of Contracts	$ (Millions)	No. of Grants	$ (Millions)
Carcinogenesis Mechanisms	4	.19	80	6.64
Chemoprevention	23	1.76	15	1.18
Molecular Carcinogenesis	21	1.60	124	11.20
Research Resources	15	1.55		
Special Projects	15	.08	104	17.16
TOTALS	78	5.18	323	36.18

Prepared May 28, 1981

TABLE II

CHEMICAL AND PHYSICAL CARCINOGENESIS BRANCH
(Extramural Activities - FY 1981)

	No. of Contracts/Grants	$ (Millions)
Research Contracts	63	3.57
Research Grants	307	33.93
Traditional Project Grants (291 grants; $26.83 million)		
Conference Grants		
New Investigator Research Grants (5 grants; $.26 million)		
Program Project Grants (11 grants; $6.84 million)		
Interspecies Comparisons (RFA)	16	2.25
Research Resources Contracts	15	1.55
Frederick Cancer Research Center	3	4.90
Research Effort (1 contract; $4.00 million)		
Extramural Resources		
Interagency Agreements (2 contracts; $.9C million)		
TOTALS	404	46.20

Prepared May 28, 1981

CHEMICAL AND PHYSICAL CARCINOGENESIS BRANCH
SUMMARY OF RESEARCH GRANTS

Overall funding for grants in Fiscal Year 1981 amounted to $36.18 million, an increase of 20% as compared with Fiscal Year 1980, but with essentially no change in the number of grants funded. The number of awards increased from 313 in Fiscal Year 1980 to 323 in Fiscal Year 1981; this includes the anticipated funding of 16 grant applications ($2.25 million) submitted in response to a request for applications (RFA) for studies on interspecies comparisons in carcinogenesis. As pertains to Traditional Project Grants (R01), 291 awards were made in Fiscal Year 1981 at a cost of $26.83 million, compared with 294 grants costing $23.54 million during the prior year. These latter data mirror a 13.9% increase in the funding of individual R01 grants. Information concerning the estimated number and total costs of the several kinds of grants funded during Fiscal Year 1981, are shown in Table II.

Table I provides information concerning the distribution of grant supported research among the several programmatic categories within the Chemical and Physical Carcinogenesis Branch. Of the $17.16 million shown for Special Projects, $6.84 million represent the cost of 11 Program Projects (P01); $4.31 million constitute the funding of 36 grants in the area of endocrine-related biochemistry of cancer; and $2.25 million represent 16 grants in the area of interspecies comparison. In the Molecular Carcinogenesis category, the emphasis is on interactions of carcinogens with biological macromolecules, and changes in cellular macromolecules and other components and in cell functions produced by chemical and physical carcinogens, cocarcinogens, and mutagens (40 grants; $3.70 million); repair of DNA damage following interaction with carcinogens (26 grants; $2.28 million); and properties of cells transformed by carcinogens (23 grants; $2.18 million). The Carcinogenesis Mechanisms category shows an emphasis (52 grants; $4.14 million) on the metabolism and physiological disposition of carcinogens and their metabolites, and the identification of proximate and ultimate forms of chemical carcinogens. The isolation, identification, and synthesis of chemical carcinogens and their metabolites, together with molecular structure-activity relationships are represented by 16 grants, at a cost of $1.34 million. Chemoprevention is largely concerned with studies on retinoids. Research Resources are supported, in the main, by contract.

Research Accomplishments

Utilizing cultures of adult rat hepatocytes on collagen gel-nylon mesh, it has been possible to demonstrate unscheduled DNA synthesis in the presence of the procarcinogen 2-acetylaminofluorene and the direct-acting carcinogen methyl methanesulfonate. Unscheduled DNA synthesis was measured as the increase in ^{3}H-methyl-thymidine incorporated into DNA in the carcinogen-treated cultures as compared with control cultures. In addition, these studies revealed that the response to the two carcinogens in cells cultured for more than 24 hours was absent unless the hepatocytes were incubated in culture medium supplemented with dexamethasone and glucagon. These studies not only indicate the potential use of the collagen gel-nylon mesh primary hepatocyte culture as an in vitro screen for chemical carcinogens but also demonstrate the importance of specific hormones in maintaining the capability for DNA repair induced by these agents in cultured hepatocytes (Sirica et al., 1980) and (Althaus et al., 1980). (P01 CA 22484).

Long-term studies (up to 84 days) have been conducted of healthy young men on very low nitrate diets. The diet is semi-synthetic and provides less than 10 mg $NaNO_3$

per day, and essentially no $NaNO_2$. Dr. S. P. Tannenbaum and co-workers observe highly individualistic patterns of excretion which reflect the dynamic behavior of nitrate in the body. They do not now know the substrates for nitrate synthesis or the source of the daily fluctuations, but every individual who has been examined demonstrates excess synthesis on low nitrate diets.

These investigators have also conducted experiments on nitrate pharmacokinetics in man using $^{15}NO_3^-$ to measure clearance (Tannenbaum et al., 1980). An individual was given 300 mg of ^{15}N-sodium nitrate and his urinary excretion monitored for up to 72 hours. The $^{15}NO_3^-$ was determined by conversion to nitrobenzene and subsequent analysis by GC-mass spectrometry of the nitrobenzene peak to determine $^{14}N/^{15}N$. The data demonstrate a fairly constant excretion of $^{14}NO_3^-$ in the period before the ^{15}N dose and in subsequent periods, while the ^{15}N shows a steady decline until it could no longer be detected at 36 to 48 hours. Thus, an oral dose of nitrate is effectively cleared from the body within 36-48 hours.

The same investigators have conducted studies with $^{15}NO_3^-$ in the conventional (SPF-derived, defined flora) and germfree rat to determine the relationship between nitrate intake and excretion (Green et al., in press). In brief, germfree and conventional rats show identical patterns of nitrate metabolism, so it is concluded that a gut flora is not essential for endogenous synthesis.

The results also show that there is $^{15}NO_3^-$ in the feces of the germfree rat and ^{15}N-urea in the urine of the conventional rat. Therefore, a major portion of the nitrate which does not appear in urine must enter the colon. In the germfree rat this nitrate remains unmetabolized and is recovered in feces, while in the conventional animal it is reduced to NH_4^+, converted to urea, and excreted as such in urine. (PO1 CA 26731).

Earlier studies from a number of laboratories showed the anti-tumor activities and mutagenicities of a number of cis-platinum (II) amine complexes, and recently Dr. J. A. Miller and associates demonstrated the carcinogenicity of cis-dichlorodiammineplatinum (II) for mice. These studies are now being extended by these investigators through examination of cis- and trans-dichlorodiammineplatinum (II) and of the three isomeric forms of dichlorodiaminocyclohexaneplatinum (II) and of aquosulfatodiaminocyclohexaneplatinum (II); the three isomeric forms differ with regard to the stereochemistry of the amine ligand.

As reported independently by other investigators, trans-dichlorodiammineplatinum (II) has very weak mutagenic activity for Salmonella typhimurium TA100. It also has no significant activity in the initiation of skin tumors in CD-1 mice or of lung adenomas in A/Jax mice. The platinum cyclohexylamine complexes also differ markedly in their mutagenic activities. Trans(+)-Dichlorodiaminocyclohexaneplatinum (II) is approximately as potent a mutagen as cis-dichlorodiammineplatinum (II) for S. typhimurium TA100 (about 200 revertants/nmole), while the corresponding sulfato derivative is about 30 per cent as active. The cis and trans (-) derivatives are each approximately 10 and 5 per cent, respectively, as active as the corresponding trans (+) analogs. Preliminary studies indicate that these latter six platinum complexes have little, if any, activity for the initiation of skin tumors in CD-1 mice. For these platinum complexes there appears to be little correlation between their mutagenicities for S. typhimurium TA100, their carcinogenicities (as determined so far) in mice, and their anti-tumor activities as reported in the literature. (Manuscript in preparation.) (CA 22484).

Relative to the question of the role of estrogen in carcinogenesis, it has been found that the 4α,5α-epoxy-17β-hydroxyestra-1-en-3-one intermediate of estradiol activation was approximately as active as 3-methylcholanthrene in the transformation of mouse fibroblast cells. The transformed cells are tumorigenic in nude mice. (Le Quesne et al., 1980) and (Purdy et al., 1981, in press). (CA 24629).

The radiometric procedure which monitors the transfer of ^{3}H from selected metabolically active sites on the estradiol molecule has the intrinsic advantage that it measures the total in vivo transformation and is independent of further metabolism, conjugation, or route of excretion. Using 17α-^{3}H-estradiol, 2-^{3}H-estradiol and 16α-^{3}H-estradiol, Dr. J. Fishman and co-workers have measured the extent of the principal transformations of estradiol in normal men and pre- and postmenopausal women. The transformations examined were oxidation of the 17β-hydroxy group and hydroxylation at C-2 or 16α. The results obtained show dramatic differences between men and women in the metabolism of estradiol, which have heretofore not been detected. Men exhibit very significantly decreased oxidation at C-17, hydroxylation at C-2 and at 16α compared to women, with the difference being greatest in the case of 2-hydroxylation. The difference in 17β-ol oxidation between men and young women is almost precisely equal to the combined deficit in 2 and 16α-hydroxylations in the male. Because no sex differences were found in the overall rates of these reactions, these results imply that in men a larger portion of the female hormone is transferred to a form or compartment where it is unavailable for these transformations and that this sequestered portion is then excreted by a non-urinary route. These results also show that of the decreased fraction of the tracer oxidized by men, proportionally less is transformed at the C-2 position than at the 16α. Thus, men form proportionately more of the uterotropic estrogens than of the CNS active catechol estrogens from the available substrate than women do. These comparative studies were carried out in the follicular stage in women. Consequently, the impact of quantitative differences in endogenous estrogen concentrations between the sexes should be minimized insofar as these metabolic changes are involved. These findings introduce a new dimension to the study of estradiol metabolism and physiology, and further studies of its implications in endocrine-related cancers have high priority.

Although there is substantial epidemiological evidence that benzene causes leukemia in humans, no animal model has yet been developed. Dr. R. E. Albert and co-workers have found some encouraging results in this regard (Snyder et al., 1978). Specifically, a small number of CD-1 mice (2 of 40) and Sprague Dawley rats (1 of 40) developed myelogenous leukemia following chronic exposure to elevated levels of benzene vapor. This is the type of leukemia often observed in humans exposed to benzene, and no reports of it occurring spontaneously in these animals have been found. In addition, 6 of 40 C57B1 mice chronically exposed to benzene vapor developed thymic lymphoma, a rare spontaneous tumor in these animals. These developments indicated that hematopoietic tumors might be produced in animals undergoing extensive inhalation exposures to benzene (Snyder et al., 1980). (PO1 CA 26724).

1'-Hydroxyestragole and 1'-hydroxysafrole are proximate carcinogenic metabolites of the naturally occurring hepatocarcinogens estragole (1-allyl-4-methoxybenzene) and safrole (1-allyl-3,4-methylenedioxybenzene). Two major (I and II) and two minor (III and IV) nucleoside adducts were found by high-performance liquid chromatography (HPLC) of enzymatic hydrolysates of hepatic DNA isolated from adult female CD-1 mice that had been given i.p. injections of [2',3'-^{3}H]1'-hydroxyestragole or [2',3'-^{3}H]1'-hydroxysafrole. Reaction of 1'-acetoxyestragole or 1'-acetoxysafrole (model

electrophilic, mutagenic, and carcinogenic esters of these two alcohols) with [^{14}C]deoxyguanosine yielded products in each case that coeluted on HPLC with Adducts I, II, and III isolated from the liver DNAs of mice given the 1'-hydroxy carcinogens. Adduct IV from the hepatic DNAs chromatographed in each case with the major adduct obtained on reaction of 1'-acetoxyestragole or 1'-acetoxysafrole and deoxyadenosine. The adducts from the hepatic DNA of mice treated with either 1'-hydroxyestragole or 1'-hydroxysafrole did not comigrate with any major reaction products of either 1'-hydroxyestragole (or -safrole)-2',3'-oxide or of 1'-oxoestragole (or -safrole). These electrophiles are metabolites of estragole (or safrole). On the basis of their chemical and physical properties, including NMR spectra, Adducts I and IV in each case were characterized as $\underline{N}^2$-(trans-isoestragol-3'-yl)-deoxyguanosine and $\underline{N}^6$(trans-isoestragol-3'-yl)-deoxyadenosine and the corresponding isosafrole derivatives. Furthermore, DNA adducts II and III derived from 1'-hydroxyestragole were tentatively characterized as $\underline{N}^2$-(estragol-1'-yl)-deoxyguanosine and $\underline{N}^2$-(cis-isoestragol-3'-yl)-deoxyguanosine, respectively. The corresponding safrole derivatives were not obtained in sufficient amounts for adequate characterization. The occurrence of these adducts in mouse liver DNA can be accounted for by reaction of a metabolically derived ester of 1'-hydroxyestragole or of 1'-hydroxysafrole with the exocyclic amino groups of the purine bases. Thus, esters of these two proximate carcinogens are apparently the major ultimate electrophilic reactants in vivo. Previous data suggest that the esters in vivo are 1'-sulfates. With both 1'-hydroxyestragole and 1'-hydroxysafrole administration, the levels of each of these four adducts in the DNA reached a maximum within 24 hours after a single dose. The removal of the adducts followed a biphasic curve with relatively rapid removal up to 3-5 days and much slower removal after this time. No preferential removal or retention of any of the adducts was observed (Phillips et al., 1981). (PO1 CA 22484).

Two responses of chick embryo fibroblasts to phorbol esters have been routinely quantitated by Driedger and Blumberg -- the decrease in fibronectin after exponential growth of the cells for three days in the presence of phorbol ester and stimulation of the rate of 2-deoxyglucose uptake 5 hours after addition of phorbol ester to cells arrested in low serum (Driedger and Blumberg, 1979), (Driedger and Blumberg, 1980a), and (Driedger and Blumberg, 1980b).

Previous studies with PMA, phorbol 12,13-dibenzoate, phorbol 12,13-diacetate, 4-O-methylPMA, phorbol 12,13,20-triacetate, and phorbol 13-acetate indicated that all the derivatives, with the exception of phorbol 12,13-dibenzoate, showed comparable maximal effects on fibronectin loss, i.e., they all possessed equal efficacy. They varied substantially, however, in the concentrations required to induce a response.

Quantitative comparison of the half-maximally effective doses (ED_{50}'s) for loss of fibronectin with those determined by Hecker for irritancy in the mouse ear (ID_{50}'s) showed excellent agreement. Over a potency range of 13,000 in the chick embryo fibroblast system, the points lay within a factor of 2 of the linear regression line between ED_{50} and ID_{50}.

A critical issue raised by the above findings was whether the correlation observed was actually one with inflammatory activity or whether it was with promotion, since these two activities generally correspond for the typical phorbol derivatives. Therefore, Dr. P. M. Blumberg and co-workers examined the activity of two classes of phorbol-related diterpene esters which had been reported to be highly inflammatory but non-promoting.

The resiniferonol derivative, resiniferatoxin, was of interest because it is the most highly inflammatory phorbol-related diterpene known. With an ID_{50} of 0.15 pmole/ear, it is 100-fold more potent than PMA for causing mouse ear inflammation (Schmidt and Evans, 1979), however, it is apparently non-promoting (Zur Hausen et al., 1979). In chick embryo fibroblasts, Driedger and Blumberg found resiniferatoxin to be 100-fold less potent than PMA in stimulating 2-deoxyglucose transport, inducing plasminogen activator, causing fibronectin loss or morphological change (Driedger and Blumberg, 1980b). The lack of activity did not reflect a species difference. Resiniferatoxin was comparably inactive on the mouse 3T3 cell line. Likewise, the lack of activity did not reflect in vitro degradation. The mouse ear assay demonstrated recovery of at least 1/2 of the inflammatory activity after incubation of resiniferatoxin for 3 days in culture medium under the conditions of the fibronectin assay. (CA 22895).

Mammary tumors have been shown to regress during postpartum lactation in rats and in women although serum prolactin levels are elevated above nonlactating levels. Prolactin has been shown to stimulate, whereas adrenal glucocorticoid hormones inhibit mammary tumor growth in rats. The present studies were conducted to determine whether the administration of dexamethasone, a potent synthetic glucocorticoid hormone, could inhibit carcinogen-induced mammary tumor growth in nonlactating rats in the presence of elevated serum prolactin produced by a dopamine receptor blocker, haloperidol. Dexamethasone alone produced significant regression of 7,12-dimethylbenz(a)anthracene-induced mammary tumors and reduced serum prolactin levels. Haloperidol alone caused significantly increased mammary tumor growth and elevated prolactin levels. When added together, a significant regression of mammary tumors resulted despite markedly elevated serum prolactin levels. Assays for the levels of specific prolactin receptors in membranes of mammary tumors revealed no significant differences in any treatment group. The results suggest that dexamethasone inhibition of mammary tumors growth is via a direct action on the tumor tissue. The inhibition is not affected by elevated serum prolactin levels produced by haloperidol and is not due to reduction of prolactin binding sites in the tumor tissue. (Aylsworth et al., 1980). (CA 10771).

Sufficient evidence exists to show that ionizing radiation is a potent human carcinogen. Little information concerning the mechanism of radiation carcinogenesis has emerged from the relevant studies in animals or in human populations. To attempt to examine mechanisms of radiation carcinogenesis, mouse embryo C3H/10T1/2 clone 8 cells (developed in the laboratory of C. Heidelberger for chemical transformation studies) were adapted for these studies. The cells represent an in vitro transformation assay system in which cellular exposure and environmental conditions can be controlled. The appearance of transformed foci following an X-ray exposure of 400-600 rads requires extensive proliferation of the cells followed by prolonged incubation under conditions of confluence. The absolute yield of transformed foci was shown to be constant over a wide range of dilutions when the progeny of irradiated cells were resuspended and replated and similar in yield to that observed in undisturbed cultures. For a given X-ray dose, the results demonstrated that the number of transformed foci per dish was independent of the number of irradiated cells plated (Kennedy et al., 1980). The observations suggest that few of the transformed clones arise as a direct consequence of the X-ray exposure and challenge the hypothesis that an X-ray-induced mutational change is responsible for the appearance of transformed foci. The results are interpreted in terms of a two-step process. The first step may involve an epigenetic change such as the induction or expression of some cell function in the surviving cells and that this change is transmitted to the progeny of the surviving cells. This change makes

them more prone to undergo the second step, transformation, when these cells are maintained under conditions of confluence. The X-ray induced functional change postulated as the initial alteration in the irradiated C3H/10T1/2 cells may be a novel example of a persistent nongenetic change in a mammalian cell line. (CA 22704).

The induction of cancer in vivo can be influenced by hormones. Of the possible mechanisms of action, it has been suggested that sex hormones may act like promoting agents in their ability to enhance cancer incidence. Mouse embryo C3H/10T1/2 cells have been used for the demonstration of two-stage (initiation and promotion) transformation in vitro with X-rays and chemical carcinogens as initiators and 12-O-tetradecanoyl-phorbol-13-acetate as the promoter. Using this cell system, the effect on the induction of malignant transformation in vitro of 17β-estradiol, given both alone and with x-irradiation, were investigated (Kennedy and Weichselbaum, 1981). Exposure of cells to 17β-estradiol alone at a concentration of 10^{-6}M for 6 weeks or 10^{-5}M for 5 days, induced malignant transformation in C3H/10T1/2 cells. When the cells were exposed to both 400 rads of X-rays and 17β-estradiol, an additive effect on transformation was demonstrated. The addition of the protease inhibitors, antipain and leupeptin, suppressed estradiol-induced transformation as well as the cocarcinogenic effect of estradiol-radiation transformation. From these results and other studies, it is suggested that the action of estradiol as a co-carcinogen may be as both a protease inducer and as an inhibitor of DNA repair. (CA 22704).

A number of in vitro and in vivo studies have demonstrated that a relationship exists between cell proliferation and chemical carcinogenesis. The frequency of neoplastic transformation is widely recognized to be directly influenced by the proliferation of target cells. However, the phase or phases of the cell cycle most susceptible to the induction of transformation by various chemical carcinogens has not been established. This would be important in helping to identify the underlying biochemical mechanisms involved in the initiation and expression of neoplasia. For this study, C3H/10T1/2 cells, synchronized by the method of release from confluence-induced arrest of growth, were exposed to different concentrations of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) for 30 minutes at various points during the cell cycle. The results demonstrate that MNNG causes a dose-dependent toxicity that increases during the first G_1 phase, reaches a maximum in early to middle S phase, and decreases during late S (Grisham et al., 1980). The rate of transformation, as measured by the formation of type III foci, increased and decreased with a similar pattern. Despite the approximate coincidence of periods of maximal toxicity and transformation with some period of the S phase, the mechanisms underlying these phenomena appear to differ. The differences demonstrated are that toxicity was linearly related to the dose of MNNG, whereas the logarithm of transformation is related to the dose of MNNG and that the ratio between toxicity and transformation varies with the cycle phase and the dose of MNNG. It is suggested that MNNG-induced toxicity results from inactivation of relatively large amounts of the genome, thus preventing successful DNA replication and cell proliferation. Transformation, however, is suggested to result from more subtle lesions which allow the DNA template to be replicated, since the expression of a transformed clone requires the repeated proliferation of the initated cell. (CA 22704).

The development of most cancers is believed to involve a multistep process in which cells progress from normal to initiated, preneoplastic, and premalignant stages to the end point of malignant neoplasia. A detailed analysis of the sequence of relevant biochemical and biological alterations associated with the development of

chemically-induced carcinogenesis is needed in order to characterize cells at each stage. A model for liver carcinogenesis has been developed which is based on the hypothesis that an initiating carcinogen induces an altered hepatocyte resistant to the cytotoxic effects of carcinogens. By the use of an appropriate selective pressure, the resistant hepatocyte can be stimulated to proliferate to produce nodules. A single administration to male F-344 rats of either diethylnitrosamine (DENA) or N-methyl-N-nitrosourea (MNU) 18 hrs. after partial hepatectomy resulted in the induction of altered hepatocytes. These altered cells were resistant to the inhibitory effect of dietary 2-acetylaminofluorene (2-AAF) on the proliferative response due to partial hepatectomy or CCl_4 administration. The induction of resistant hepatocytes was considered to represent initiation of liver carcinogenesis in this model. Since resistant hepatocytes persisted for up to 36 weeks with no decrease in their number, the induction of functional resistance is considered to be an irreversible early step in hepatocellular carcinogenesis (Solt et al., 1980). (CA 21157).

The functional resistance to the cytotoxic effects of carcinogens shown to be induced in hepatocytes by exposure to a number of hepatocarcinogens (DENA, aflatoxin B_1, 2-AAF) can also be induced by a non-hepatocarcinogen, MNU, if given in association with a proliferative stimulus. Since compounds known to be non-hepatocarcinogens such as urethan, 7,12-dimethylbenz(a)anthracene (DMBA), MNU and 3-methylcholanthrene (3-MC) can induce liver cancer in adult rats and mice if given in association with partial hepatectomy or a single necrogenic dose of CCl_4, several other hepato- and non-hepatocarcinogens were examined to see if they also induce functionally resistant hepatocytes. The 21 different chemical carcinogens used included 5 polycyclic aromatic hydrocarbons, 5 aromatic amines, 4 N-nitroso compounds and 7 miscellaneous compounds such as urethan, safrole, and dieldrin. As controls, 7 different noncarcinogenic analogs were also tested. All chemical carcinogens tested were observed to induce resistant hepatocytes. Foci of these cells are readily visualized histochemically by staining for gamma-glutamyl transpeptidase (GGT) activity. (Tsuda et al., 1980). Thus, the results suggest that one of the major determinants for carcinogenic activity of many compounds for the liver may be the presence or induction of cell proliferation at an appropriate time relative to the time of administration of the compound and that this model system can be developed as an in vivo short-term test system for carcinogens. Before being generally useful, it was noted that of several necessary improvements in the design of the system, an initial one would be the replacement of the dietary 2-AAF used for selection of resistant cells by a noncarcinogen. (CA 25094).

Studies conducted by Carr and Laishes, 1981, were designed to identify compounds that might be selectively toxic to normal hepatocytes while not affecting carcinogen altered hepatocytes. Primary cultures of hepatocytes isolated from livers of normal, partially hepatectomized, and 2-AAF-treated rats were used. Cell survival in culture was measured in the presence of various dose levels of test compounds, all of which had known antiproliferative action. Normal rat liver cells, but not carcinogen-treated cells, were found to be differentially sensitive to the toxic effects of methotrexate, adriamycin, and cycloheximide. Adriamycin, but not cycloheximide, was also toxic to cells from partially hepatectomized rats. Carcinogen altered cells were shown to be resistant to several other chemicals which are used in the chemical treatment of cancer. Thus, non-hepatocarcinogens such as methotrexate, adriamycin and cycloheximide may be suitable substitutes for 2-AAF in the hepatocarcinogenesis model system described by Solt et al., 1980. Primary monolayer cultures may also be a useful system for the quantitative in vitro study

of the mechanisms by which cancer cells acquire resistance to chemotherapeutic agents. (CA 24818).

An early event associated with liver carcinogenesis is described by Blackburn et al., 1980. The administration of three different liver carcinogens (2-AAF, 3'methyl-4-dimethylaminoazobenzene, and ethionine) to rats results in an early and direct interaction of these carcinogens with a specific polypeptide. The interaction is shown to result in a marked reduction of both the carcinogen:polypeptide complex and the amount of free polypeptide itself. The specificity of the interaction appears to reside in the target polypeptide itself which is shown to have a molecular weight of 14,700. The investigators speculate that this polypeptide may have a growth regulatory function since its size is near that of known polypeptide growth regulators. (CA 05945).

Carcinogenic N-alkyl-N-nitroso compounds have been shown to cause a rapid decrease in the total size of the NAD pool in various cells. This rapid turnover of NAD could result from either an inhibition of biosynthesis, an enhancement of degradation or both. In order to characterize the mechanism of NAD lowering by N-alkyl-N-nitroso compounds, NAD metabolism was studied in mouse 3T3 cells with N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) as a model compound. The addition of 130μM MNNG to the cultures resulted in a 50% reduction in the NAD pool in 1 hr without affecting the NADP or ATP pools (Jacobson et al., 1980). The rate of biosynthesis of NAD was not affected by 140μM MNNG, demonstrating that an increased rate of degradation causes the lowering of NAD. No change in the activity of NAD glycohydrolase results from the treatment of cells with 340 μM MNNG for 30 min, but the activity of the other known NAD degradative enzyme, poly(ADP-ribose) polymerase, increased 7-fold. The lowering of NAD was shown to be coincident with the appearance of alkali-labile sites in DNA. These studies provide evidence that the acute lowering of NAD in cells by N-alkyl-N-nitroso compounds is due to an increased synthesis of poly(ADP-ribose) which occurs in response to DNA damage. To gain further evidence for the specific relationship between DNA damage and acute depression of cellular NAD pools, the effect of several classes of DNA damaging chemicals on NAD metabolism was examined in freshly isolated human lymphocytes (Rankin et al., 1980). Each of the direct DNA damaging chemicals tested (MNNG, methyl methanesulfonate, N-acetoxy-2-acetylaminofluorene, 7-bromomethylbenz(a)-anthracene, benzo(a)pyrene-7,8-diol-9,10-epoxide and benzo(a)pyrene-4,5-epoxide) caused a rapid lowering of cellular NAD pools. 2-Acetylaminofluorene and 13 polycyclic aromatic hydrocarbons and derivatives that are non-DNA damaging or require metabolic activation to become DNA-damaging did not cause lowering of NAD. Thus, the results show that the lowering of NAD pools is highly correlated to DNA damage. The lowering of NAD levels may be closely related to the DNA repair process. Lymphocytes taken from different donors were observed to vary in the effect of DNA damaging agents on NAD levels. The possibility was raised that individual variations in rate of lowering of NAD levels may be related to DNA repair capability. It is suggested that this methodology may be useful in studying factors related to DNA repair capability in the human population. (CA 23994).

The initiation of the carcinogenic process by chemical carcinogens appears to involve alterations in DNA structure and/or its subsequent replication as an obligatory first step. The various bacterial and mammalian mutagenesis assays used to identify potential carcinogens are based on measuring the consequences of such alterations. A number of metals exhibit activity in mutagenesis systems and are carcinogenic in experimental animals. Epidemiological studies have identified arsenic, selenium and chromium as being particularly significant with regard to

carcinogenesis in humans. Previous studies had indicated that metals can alter the fidelity of DNA synthesis and that the enhanced infidelity correlates with the mutagenicity and/or the carcinogenicity of the metal. Thus, it was hypothesized that the underlying mechanism of metal mutagenesis might be the result of infidelity of DNA replication. To test this hypothesis, an in vitro DNA synthesizing system using poly [d(A-T)] or ΦX174 DNA as the template and purified E. coli DNA polymerase I was used and the frequency of incorporation of noncomplementary nucleotides is measured (Tkeshelashvili et al., 1980). The addition of arsenic or selenium to the test system showed that it did not significantly alter the accuracy of poly [d(A-T)] replication under the normal conditions of magnesium activation. The mutagenic activity of manganese was also not affected by the two metals. The presence of chromium resulted in diminished fidelity with the misincorporated bases present as single-base substitutions. The results show that the frequency of such base substitutions in vitro is much greater than the observed mutation rates in cells, suggesting the presence of other protein factors in cells which enhance the accuracy of DNA replication. Further analysis of the contribution of each component of the DNA replication apparatus to the overall accuracy should lead to a determination of whether the underlying mechanism of metal mutagenesis is infidelity during DNA replication. (CA 24998).

Pharmacological agents that interact noncovalently with DNA were also studied using the in vitro infidelity assay (Sirover and Loeb, 1980). Several steroids such as diethylstilbestrol and estradiol, intercalating and chemotherapeutic agents such as adriamycin and daunomycin caused an increase in the relative frequency of incorrect deoxynucleotide incorporation. Thus, noncovalent interactions of compounds with DNA, even though they are comparatively unstable and presumably of short duration, can affect the fidelity of DNA synthesis. (CA 24998).

A decrease in the fidelity of DNA replication following exposure to chemical carcinogens may result from the formation of an altered DNA polymerase molecule as well as from damage to the DNA template. The appearance of a significant degree of infidelity in the function of DNA polymerase-α isolated from rat liver cytoplasm following exposure to N-2-acetylaminofluorene (2-AAF) was previously noted. The other DNA polymerases, nuclear polymerase α, and nuclear and cytoplasmic DNA polymerase β, remained unaltered. To identify the mechanism of infidelity, the purification schemes for the DNA polymerases were modified to obtain highly purified enzymes. The identification and separation of a subspecies of cytoplasmic DNA polymerase-α, polymerase-α_1, from the normal component, polymerase-α_2, resulted (Chan and Becker, 1980). The results show that polymerase-α_2 exhibited normal fidelity throughout the purification procedure while polymerase-α_1, (purified 10,250-fold) continued to exhibit a severe degree of infidelity. However, its relatively small quantity (about 5-10% of the total DNA polymerase activity) and a question as to its location in situ make it impossible to suggest any role in the evolution of hepatocarcinogenesis. (CA 20657).

The metabolic activation of carcinogenic arylamines to reactive derivatives is a complex process which appears to involve acetylation, N-hydroxylation and esterification. The rate-limiting step proposed for the activation process is N-hydroxylation, which is the initial activation step for the acetylated arylamine carcinogen 2-acetylaminofluorene (2-AAF). Aromatic hydroxylation, however, appears to lead to detoxification products. Both types of reactions are catalyzed by the cytochrome P-450 monooxygenases which consist of multiple forms of cytochrome P-450. Four highly purified forms of rabbit liver microsomal cytochromes P-450 were shown to catalyze the N- and ring-hydroxylation of 2-AAF at different rates (Johnson

et al., 1980). The major cytochrome P-450 form induced in adult rabbits by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), Form 4, catalyzed the N-hydroxylation of 2-AAF to the extent of 70% of all the metabolites formed and at a greater rate than the other three forms. Form 3, a constitutive form, and Form 6, the major TCDD-inducible form in neonatal rabbit liver, metabolized 2-AAF at one half the rate as Form 4 and produced predominantly phenols (90% of all metabolites). No metabolic activity was exhibited by Form 2, the major phenobarbital-inducible cytochrome P-450 form. Thus, the differential occurrence of the various cytochrome P-450 forms in the target organ should affect the balance between the activation or the detoxification pathway of 2-AAF metabolism and possibly the carcinogenicity of 2-AAF. (CA 24146).

The initial N-acetylation of arylamine drugs and carcinogens is catalyzed by an enzyme, N-acetyltransferase, which is found in liver cytoplasm. The activity of the enzyme is genetically polymorphic in some species such as man and rabbits and individuals in the population are distributed bimodally into rapid and slow acetylators of arylamine compounds. Thus, in man, differences in drug toxicity are associated with differences in the acetylator phenotype. Following acetylation and N-hydroxylation, a subsequent necessary step in the activation pathway of arylamines involves the enzymatic removal of the N-acetyl moiety of the hydroxamic acid to form a derivative that can react spontaneously with nucleic acid and proteins. The enzyme responsible is believed to be an arylhydroxamic acid N,O-acyltransferase. Since both the above-mentioned enzymes catalyze an N-acetyl transfer and may be involved in arylamine tumorigenesis, a comparative biochemical and genetic study was undertaken (Glowinski et al., 1980). Both enzyme activities were shown to be high in rapid acetylator rabbits and low in slow acetylator rabbits. This indicated that these two acetyl transfer steps in the metabolic activation of the arylamines studied (aminofluorene and sulfamethazine) are under common genetic control. The purification of the two enzyme activities through several steps and ending with polyacrylamide gel electrophoresis failed to resolve the two activities. The results obtained strongly suggest that the N-acetyl transferase reaction and the intramolecular N,O-acetyl transfer by arylhydroxamic acid N,O-acyltransferase are properties of the same enzyme. If the results obtained in rabbits are also true for man, the broad base of data on human genetic differences in N-acetylation of arylamine drugs could possibly be extended to predict individual differences in susceptibility to cancer from carcinogenic arylamines. (CA 23386).

Studies concerning the metabolism of polycyclic aromatic hydrocarbons led to two reports from the same laboratory on enzyme catalyzed conversions of diol epoxides into glutathione conjugates. In the first (Cooper et al., 1980a), it was shown that incubation of anti-benz(a)anthracene-8,9-diol 10,11-oxide with dialyzed rat-liver supernatant and glutathione led to formation of a glutathione conjugate of anti-benz(a)anthracene-8,9-diol 10,11-oxide. The enzyme involved appears to be glutathione S-transferase. These results appear to provide the first clear evidence that a diol-epoxide formed from a polycyclic aromatic hydrocarbon by metabolism, can act as a substrate for glutathione S-transferase. The second paper (Cooper et al., 1980b) describes the enzyme-catalyzed conversion of anti-benzo(a)pyrene-7,8-diol 9,10-oxide into a glutathione conjugate using the same system described in the previous paper. Conjugate formation was minimal on the absence of either glutathione or rat-liver supernatant. Data obtained also indicate that formation of the glutathione conjugate is dependent on the presence of intact anti-benzo(a)-pyrene-7,8-diol 9,10-oxide and that hydrolysis products formed from this diol epoxide are not substrates for glutathione S-transferase. The authors hypothesize that the enzyme-catalyzed conversion of these diol-epoxides into other metabolites including glutathione conjugates might, at least in part, control the extent of

binding of benzo(a)pyrene to cellular constituents. This hypothesis is consistent with the results of earlier studies where, when either rat-liver supernatant fractions or glutathione and glutathione S-transferase were included in incubation mixtures, the level of binding of the diol-epoxide to DNA decreased. (CA 21959).

The metabolism of benzo(a)pyrene was compared under culture conditions used in two short-term assays, cell transformation and cell-mediated mutation (Baird et al., 1981). In both of these assays, which are used to detect carcinogens, the hydrocarbon was activated by early-passage embryo cells from two different species, rat and hamster. The results reported demonstrate that the metabolites formed from a hydrocarbon such as benzo(a)pyrene depend not only on the source of the cells but on the density of the cell culture. Under low density conditions analogous to those in the transformation assay, the two-cell types released both benzo(a)pyrene-phenols and benzo(a)pyrene-phenol glucuronides into the culture medium. Only conjugated benzo(a)pyrene phenols were released into the culture medium under the high density conditions of the cell mediated mutation assay. The authors caution that in order to relate the pathways of hydrocarbon metabolism to the induction of specific biological effects, metabolism must be measured under exact conditions of the short-term assay being used. (CA 08936).

Continuing their work on foreign body tumorigenesis, Brand and Brand (1980) attempted to assess the risk of development of foreign body related cancers in humans in two ways. First, available case reports were collected and evaluated with special attention to the recorded latencies. Second, 27 specimens of chronic human foreign-body reactive tissues were screened using a method developed in mice that permits identification of preneoplastic cells. Analysis of available case reports indicated that well over 25% appeared approximately with 15 years of implantation and 50% within 25 years. The present upsurge of medical and cosmetic implantation began during the late 1950s and early 1960s. Based on the analysis of case reports, at least 25% of implant-related cancers should have emerged by now. Very few cases have been reported in recent years. Thus, the authors conclude with reasonable assurance that the incidence of cancer arising at implantation sites will remain minimal.

The screening method which involved culturing tissue from the 27 specimens, did not yield any abnormal clonally aneuploid cells resembling the precancer cells of murine foreign body reactions. Various established techniques used to stimulate the precancer cell type were unsuccessful. In addition, the authors observed that human fibroblasts differed from mouse fibroblasts by much more vigorous proliferation and cultured persistence. When human and murine foreign body reactive tissues were cultured separately and in variously proportioned mixtures, mouse precancer cells were detected only when mouse tissue was cultured separately. Thus, the authors conclude that proliferating human fibroblasts do inhibit the establishment of murine foreign body reaction precancer cells.

In summary, the authors state that the rarity of human foreign body cancers can satisfactorily be explained by the rare emergence of precancer cells in human foreign body reactions. Also, human fibroblasts, which easily overpower murine precancer cells in culture, may provide an additional safeguard by suppressing potential precancer cells emerging in human foreign body reactions. (CA 10712).

Esophageal cancer in humans occurs worldwide with the highest rates reported from China, areas of Iran and the Soviet Union, and the Transkei of South Africa. Several environmental and dietary factors have been implicated as etiological agents

of human esophageal cancer. Among these are certain nitrosamine chemicals that occur in the diet and in tobacco smoke, and which have been isolated from the urine and feces of humans.

Most experimental studies on the chemical induction of esophageal cancer were done in rats. Several nitrosamine chemicals produce esophageal cancer in rats however,the mechanism(s) through which these compounds change normal esophageal epithelial cells to cancer cells is unknown. Recently, Dr. G. D. Stoner's laboratory developed an in vitro model for investigation of nitrosamine-induced esophageal cancer. Tissue fragments of rat esophagus were treated in culture with several concentrations of the potent esophageal carcinogen, N-nitrosomethyl-benzylamine (MBN). Epithelial cell lines have been established from MBN-treated cultures but not from the controls. After a period of 6-9 months in culture, MBN-treated cell cultures acquired the ability to grow in soft agar and to produce well-differentiated squamous cell carcinomas after transplantation into appropriate hosts. These carcinomas are essentially identical in appearance to squamous cell carcinomas in the esophagus of rats in vivo and in the human esophagus. Studies are underway to determine: (a) if other carcinogens such as asbestos change normal esophageal epithelial cells to cancer cells (asbestos is a human esophageal carcinogen); (b) if promoting agents influence the frequency of cell transformation; and (c) the molecular interactions between nitrosamine compounds and esophageal epithelial cells. (CA 28950).

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SUMMARY REPORT
CARCINOGENESIS MECHANISMS

Included under Carcinogenesis Mechanisms are activities relating to metabolism, toxicity and physiological disposition of carcinogens and their metabolites; mechanisms of action; identification of proximate and ultimate carcinogenic forms; carcinogen-mutagen relationships; isolation, identification, and synthesis of known and suspect chemical carcinogens and their metabolites; and molecular structure activity relationships. The number of active contracts in this area has been significantly reduced over the last fiscal year. Most of the contracts support basic research. Thus, in accordance with the National Cancer Institute's policy to phase out basic research contracts, all of the contracts in this area are being brought to orderly conclusion at their termination dates. Three of the four remaining contracts will end during FY 81 and the fourth will end in FY 83.

All of the active contracts address the metabolism and physiological disposition of carcinogens and their metabolites. Two are conducting studies of the metabolic capacity in intestinal mucosa. At the American Health Foundation (NO1-CP-75948), studies continue on the tissue specific metabolism of selected carcinogens known to produce tumors of the large bowel in mice, rats, and hamsters. Alcohol dehydrogenase isozymes were isolated from rat liver and from rat colon mucosa, and their activity towards methylazoxymethanol was compared. In spite of earlier promising results, further experiments indicate that while alcohol dehydrogenase may play a role in the metabolism of methylazoxymethanol, it does not by itself, determine the organotropism of this carcinogen. A membrane bound enzyme complex consisting of phosphoribosylpyrophosphate synthetase and hypoxanthine phosphoribosyltransferase was found in rat intestine at Sloan Kettering Institute for Cancer Research (NO1-CP-75950). This complex, which appears to act as a transport system for taking 6-hydroxy-purines from the intestinal lumen and incorporating them into the cytoplasm as ribonucleotides, has been shown to be disturbed by carcinogen exposure.

Metabolism of six carcinogens is being studied at Southern Research Institute (NO1-CP-55721). Examples of studies performed include elucidation of metabolic pathways, identification of metabolites, and macromolecular binding.

The kinetics of metabolism of carcinogenic 2-hydroxybenzo(a)pyrene are being compared with those of the non-carcinogenic positional isomeric phenols, 3-hydroxybenzo(a)pyrene and 9-hydroxybenzo(a)pyrene at Oak Ridge National Laboratories (YO1-CP-90204). In hamster embryonic cells, the non-carcinogenic phenols are metabolized to water-soluble derivatives much more rapidly than the 2-hydroxybenzo(a)pyrene. It appears that 2-hydroxybenzo(a)pyrene follows a kinetic pathway in cell culture that is identical to that of the parent benzo(a)pyrene.

CARCINOGENESIS MECHANISMS

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Department of Energy Oak Ridge National Laboratory (YO1-CP-90204) (Formerly YO1-CP-50200)	Mechanisms of Action of Chemical Carcinogens	1431
Sloan-Kettering Institute For Cancer Research (NO1-CP-75950)	Studies of Metabolic Capacity in Intestinal Mucosa	1433
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AMERICAN HEALTH FOUNDATION (NO1-CP-75948)

Title: Studies of Metabolic Capacity in Intestinal Mucosa

Contractor's Project Director: Dr. Emerich S. Fiala

Project Officer (NCI): Dr. Marcia D. Litwack

Objectives:
1. Determine, using germfree and conventional rats, the metabolic activity of the colonic microflora to metabolize a wide variety of precarcinogens, carcinogens and their metabolites, like 1,2-dimethylhydrazine, azoxymethane, methylazoxymethanol, 3,2'-dimethyl-4-aminobiphenyl and 3-methyl-2-naphthylamine and the metabolic capacity of the small intestinal and colonic mucosa to metabolize these compounds and their metabolites to the proximate and/or ultimate carcinogens.

2. To incubate the radiolabelled compounds with the colonic contents, small intestinal and colonic mucosal fractions, and isolated small intestinal and colonic loops from the germfree and conventional rats and the metabolic products formed will be identified.

3. To investigate the metabolic capacity of the liver and kidney in the germfree and conventional rats to metabolize the above precarcinogens and carcinogens to ultimate or proximate carcinogens and compare with the values obtained from the intestinal and colonic mucosa.

Major Findings: Crystalline horse liver alcohol dehydrogenase in the presence of NAD was demonstrated to metabolize methylazoxymethanol (MAM) to methanol, formaldehyde and an unidentified species binding to NAD and/or NADH. However, enormous concentrations (e.g., 5 mg/ml) of enzyme were necessary before a measurable rate of this reaction could be observed. Using cytosols from rat liver and rat colon mucosa, the reaction was observed with the former but only just barely with the latter. These reactions were sensitive to pyrazole inhibition.

In another approach, histochemical methods were utilized to determine ADH in rat organs and in subcellular sites in the colon mucosa. The enzyme was found not to be preferentially localized in tissues sensitive to MAM carcinogenicity but rather its distribution was ubiquitous. The colon mucosa contained much less ADH than the liver, and the enzyme was localized in the upper portions of the crypts. Also, colon mucosa from the guinea pig, a rodent species in which MAM does not induce colon tumors, contained as much or more colon mucosal ADH than the rat, hamster or mouse, rodent species which are highly sensitive to MAM colon carcinogenicity.

In further work, DEAE cellulose chromatography was used to isolate ADH isozymes from rat liver and from colon mucosa so that their activity towards MAM could be compared. In colon mucosa, electrophoretic anodal band B_2

showed activity with ethanol and hexenol but not with MAM. Anodal band B_1 likewise showed activity with both ethanol and hexenol but not with MAM. In contrast, cathodal band A, a minor colon ADH isozyme compared to B_1 and B_2, showed activity with ethanol, hexenol as well as MAM. However, isozyme A was also the major rat liver ADH isozyme. Thus, the contractor concluded that while ADH may play a role in the metabolism of MAM, it does not, by itself, determine the organotropism of this carcinogen.

To determine the relative ability of the liver and colon to activate 3,2'-dimethyl-4-aminobiphenyl (DMAB), its metabolism was studied in primary hepatocyte culture (PHC) and in colon explants (CE) obtained from F344 rats.

In contrast to rat liver microsomal subfraction, no free N-oxidized (C-nitroso- or N-hydroxy-DMAB) metabolites of ^{3}H-DMAB were detected following incubation in PHC. However, the hepatocytes readily oxidized and conjugated over 90% of the ^{3}H-DMAB present (10^{-5}M) in 18 hours of incubation, releasing sulfate and glucuronide conjugates (>80%) and N-acetyl-DMAB (~4%) into the medium. Isolation and analysis of the conjugated metabolites by Sephadex LH-20 chromatography and by HPLC revealed that N-glucuronides were formed to a large extent (27%). The LH-20 fraction containing N-glucuronides was further separated into two radioactive peaks by HPLC. Upon mild acid treatment (pH 5, 37°, 180 min) the major component (~90%) was completely hydrolyzed to DMAB, and glucuronic acid. The minor peak on acid hydrolysis, yielded C-nitroso-DMAB and an acid-stable conjugate in about equal amounts.

Studies with rat CE revealed a number of significant differences compared to that of PHC. CE metabolized ^{3}H-DMAB largely to N-acetyl-DMAB (~15%) and only about 5% acid conjugates. The conjugate peak consisted of N-glucuronides of DMAB (~90%) and N-hydroxy-DMAB. Acid hydrolysis of the latter gave C-nitroso and azoxy derivatives of DMAB.

Significance to Biomedical Research and the Program of the Institute:
1,2-Dimethylhydrazine and its chemical derivatives such as azoxymethane and methylazoxymethanol possess high specificity in producing tumors of the large bowel in mice, rats and hamsters. Such organotropism is also shown by 3,2'-dimethyl-4-aminobiphenyl and 3-methyl-2-naphthylamine although to a much lesser degree. The working hypothesis of the contract is that tissue specific metabolism is a primary factor in the mechanism of organotropism of chemical carcinogens. Since it is likely that cancers of the large bowel and other organs in the humans owe their etiology to analogous chemical carcinogens, perhaps endogenously produced, it is important to determine critical steps in carcinogen activation and detoxication and to evaluate to what extent these occur in target and non-target tissues.

Proposed Course: This contract will expire May 29, 1981.

Date Contract Initiated: September 30, 1977

Current Annual Level: 0

DEPARTMENT OF ENERGY/OAK RIDGE NATIONAL LABORATORY (YO1-CP-90204)(Formerly YO1-CP-50200)

Title: Mechanisms of Action of Chemical Carcinogens

Contractor's Project Director: Dr. James K. Selkirk

Project Officer (NCI): Dr. Marcia D. Litwack

Objectives: To study the comparative tissue and cell activation/detoxication of polycyclic aromatic hydrocarbons in potential target tissues of rodent model systems, with particular emphasis on benzo(a)pyrene and its metabolites and analogs.

Major Findings: During the past year, efforts were concentrated mainly on 2-OH-B(a)P in terms of the kinetics of metabolism versus non-carcinogenic positional isomeric phenols 3-OH-B(a)P and 9-OH-B(a)P which are both metabolic intermediates in all known eukaryotic systems. The non-carcinogenic phenols become water-soluble derivatives as detoxification products by hamster embryonic cells much more rapidly than the carcinogenic 2-OH-B(a)P which follows a kinetic pathway identical to the parent benzo(a)pyrene in the cell culture systems. By separating both intracellular and extracellular metabolites, a single major metabolite for 2-OH-B(a)P in hamster embryonic cells has been found. However, at least one additional metabolite, co-chromatographing in the same region, has been found in epidermal cells, both rodent and human, suggesting that they follow more complicated metabolic pathways in epidermal cells. Metabolite I in hamster embryonic cells has been isolated characterized by ultraviolet fluorescence and mass spectroscopy as a diphenolic product, although at the present moment the region of the ring where the secondary phenolic group occurs has not been determined. Sufficient amounts of metabolite II from both rodent and human skin cells are currently being isolated to undergo similar chemical characterization as with metabolite I.

Since metabolism in vivo is directed toward non-toxic water-soluble products, the rate of 2-OH-B(a)P in cell culture into its final detoxification products has been pursued in order to understand the overall kinetics of metabolism.

Separation and Identification of Water-soluble Metabolites of 2-OH-B(a)P -- After a 48-hour incubation with HEF cultures, approximately 70-80% of ^{3}H-2-OH-B(a)P is converted to water-soluble metabolites. Of these, 50-60% are glucuronic acid conjugates, which can be broken down by β-glucuronidase to release mostly parent 2-OH-B(a)P. This leaves 30-40% of the original radioactivity in the form of unidentified aqueous derivatives. Techniques to fractionate these compounds have therefore been developed. Samples of HEF culture medium containing ^{3}H-2-OH-B(a)P metabolites were extensively extracted with ethyl acetate, and the medium with the remaining aqueous 2-OH-B(a)P derivatives was loaded onto Bio-gel P-2 columns (25 cm x 2.6 cm i.d.) and eluted with water. Three distinct peaks of radioactivity were collected from this system. This separation is comparable to that found on Sephadex LH-20 columns, using water to elute two peaks, then methanol elution for a third peak. The three peaks obtained are roughly equivalent in both chromatography systems.

Attempts have been made to identify the metabolites found in each peak from the LH-20 system. The first peak elutes at the exclusion volume to the column, and contains large amounts of protein, as indicated by TCA precipitation and fluorescamine assays. Polyacrylamide gel electrophoresis indicates that the major component of this fraction is bovine serum albumin, suggesting that this peak may largely represent 2-OH-B(a)P binding to serum protein.

The third peak, which elutes from the LH-20 column with methanol, contains no detectable protein, and has fluorescence spectra very similar to parent 2-OH-B(a)P, with a 2-5 nm shift in the major peaks. However, this metabolite fraction contains no ionizable phenolic group, as evidenced by the lack of any shift in fluorescence with addition of NaOH. Treatment of this peak with β-glucuronidase results in >95% release of the radioactivity into organic-extractable forms, mostly 2-OH-B(a)P. Peak 3 then, appears to be a purified glucuronic acid conjugate of 2-OH-B(a)P itself.

The identity of the middle peak eluted from the LH-20 system is less clear, although present evidence suggests that a glutathione conjugate may account for at least some of the radioactivity. Sulfatase and β-glucuronidase treatment have no cleaving effect, and the peak is positive for protein by the fluorescamine assay, but not TCA precipitation. Free glutathione, as well as protein, do not elute with this peak from the LH-20 column. An assay system to measure the presence of glutathione or its conjugates in this system has been adapted. Samples are first treated with γ-glutamyltransferase to release glutamic acid from any glutathione present, then the amount of free glutamic acid is measured using glutamate dehydrogenase and NAS. By this method, the presence of glutathione, possibly in conjugated form, was detected in peak 2, but not in molar quantities sufficient to account for all of the 2-OH-B(a)P derivatives within the peak. An attempt is currently being made to further fractionate peak 2 to determine if there is more than one substance present. Analysis of the type of chemical derivative this compound is, including the region of the molecule derivatized, may be critical to understanding the carcinogenicity of 2-OH-B(a)P. In addition, it is hoped to gain insight into the structural and physical requirements of both B(a)P and this phenolic isomer that contribute to their carcinogenic activity.

Significance to Biomedical Research and the Program of the Institute:
At the current moment, there is no mechanism of action for any carcinogen in any species. The work on this project attempts to understand the total processing of metabolic activation of environmental carcinogens. Understanding the steps of activation and detoxification for any chemical carcinogen, including the activated intermediates in the critical sites for alkylation, will help determine how the biochemical processes involving malignant transformation by chemicals can be altered.

Proposed Course: Continuation of studies with specific carcinogenic probes utilizing comparative biochemistry between human and rodent cells in tissue culture is proposed. Since the main goal of this project is the understanding of chemical carcinogenesis in humans, it is hoped to gain some knowledge of the biochemical variance between human and rodent and also between susceptible and resistant rodent cells.

Date Contract Initiated: April 1, 1979

Current Annual Level: $192,056

SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH (NO1-CP-75950)

Title: Studies of Metabolic Capacity in Intestinal Mucosa

Contractor's Project Director: Dr. M. Earl Balis

Project Officer (NCI): Dr. Marcia D. Litwack

Objectives: This is a program designed to ascertain the interactions among the many factors that contribute to colon cancer. A considerable body of data has been acquired that indicates a role of diet, reducing agents, and endogenous or exogenous chemicals on the incidence of the disease. It is the goal of this program to identify the significance of each factor and their interdependence. The methodology is to see if changes brought about by known carcinogens can be used as criteria of carcinogenic potency of other stresses. It is further hoped that assay of relative changes in biochemical parameters, in the dividing and differentiated cells, will provide a useful screen for potential colon carcinogens. Emphasis will be placed on early changes. Success in this would permit a simple means of detecting potential carcinogens and effectors of other carcinogens.

Major Findings: There appears to be a membrane-bound enzyme complex that consists of phosphoribosylpyrophosphate (PRPP) synthetase and hypoxanthine phosphoribosyltransferase (HPRTase) in the intestines. This complex appears to act as a transport system for taking 6-OH purines from the lumen and incorporating them into the cytoplasm as ribonucleotides. This unit has been shown to be disturbed by carcinogen exposure.

Changes in polyamine metabolizing enzymes indicated that diamine oxidase as well as the enzymes of polyamine synthesis are really reflectors of carcinogenesis. Some of the changes in ornithine decarboxylase were thought to have possibly been due to specific proteolysis of the enzyme in normal colon. It has been shown that this is not the case. However, an inhibitor of the enzyme has been shown to be present. This is not found after exposure to colon carcinogens. Somehow, as an early step in colon carcinogenesis, the synthesis of this compound is inhibited.

The coexistence of high levels of putrescine and ODC in DMH-induced colon tumors may be explained by the inability of these tumors to produce the ODC inhibitor in the presence of high levels of putrescine Diamine oxidase, an enzyme that destroys putrescine, was found to be very low in descending rat colon, where tumors are most readily induced; and it is very low in large bowel tumors.

Significance to Biomedical Research and the Program of the Institute: These studies indicate a possible basis for the design of immunochemical tests capable of detecting early tumors of the colon and perhaps other organs. There is also a possibility that premalignant cells can be detected. This could be

applicable to the examination of colonic brushings. The detection by a very sensitive technique of aberrant cells in the so-called normal tissue surrounding tumors may be of great prognostic value.

The explanations of tumor-specific and tumor-induced enzyme changes may permit a rational design of drugs capable of inhibiting tumors of the large bowel.

Proposed Course: This contract ended December 29, 1980

Date Contract Initiated: September 30, 1977

Current Annual Level: 0

SOUTHERN RESEARCH INSTITUTE (NO1-CP-55721)

Title: Metabolism of Carcinogenic Compounds

Contractor's Project Director: Dr. Donald L. Hill

Project Officer (NCI): Dr. Elizabeth Weisburger

Objectives: To synthesize, or otherwise acquire, six designated carcinogens labeled with carbon-14, and to study the metabolic pathways of these compounds in rats in attempts to determine their mechanism of action.

Major Findings: In studies with enzymes of cell-free preparations from rat liver, it was found that radioactivity from [^{14}C]1,2-dibromoethane binds preferentially to added polycytidylic acid when the cell-free system contains cytosol and glutathione. The radioactivity binds selectively to microsomal protein, however, when the cell-free system contains microsomes and TPNH. The binding to microsomal protein is inhibited by agents that inhibit microsomal oxidase, and the binding to polycytidylic acid is inhibited by compounds that are substrates for glutathione S-transferase. The interpretation of this information was that metabolites with different reactive properties are produced in the two systems.

A mono-oxygenated derivative of 4-chloro-2-methylaniline has been found in a neutral extract of the urine of dosed rats. Chemical synthesis of the hydroxymethyl and carboxylic acid derivatives of 4-chloro-2-methylaniline enabled the contractor to demonstrate that the metabolite is neither of these compounds. The metabolite appears to be a ring-hydroxylated derivative. A different extraction procedure, liquid chromatography with a variety of solid phases, and gas chromatography are being evaluated in an attempt to derive metabolites of 4-chloro-2-methylaniline of sufficient purity for mass spectral analysis.

In a microsomal system, radioactivity from [methylene-^{14}C] reduced Michler's ketone becomes bound to macromolecules present in the system. The reaction is dependent upon NADPH, and the rate increases when microsomes from phenobarbital-pretreated rats are used.

Major metabolites of Michler's ketone appearing in the bile of rats are the unsymmetrical, didemethylated derivative; the tetrademethylated, monoacetylated

derivative; and the tridemethylated derivative. Various products, monohydroxylated on the ring, are also minor metabolites.

Chemical syntheses of N,N'-diacetyl-4,4'-methylenedianiline, N,N,N'-trimethyl-N'-acetyl-4,4'-methylenedianiline, and N-acetyl-4,4'-methylenedianiline have been achieved. These compounds, possible metabolites of reduced Michler's ketone, were not identical to any of the major metabolites appearing in the urine of rats given oral doses of this compound.

Some minor biliary metabolites of reduced Michler's ketone have been identified as N,N-dimethyl-N'-acetyl-4,4'-methylenedianiline, N,N'-diacetyl-4,4'-methylenedianiline, N-methyl-N,N'-diacetyl-4,4'-methylenedianiline, N-methyl-N'-acetyl-4,4'-methylenedianiline (or N-methyl-N-acetyl-4,4'-methylenedianiline), and a ring-hydroxylated derivative of N-methyl-N,N'-diacetyl-4,4'-methylenedianiline.

Significance to Biomedical Research and the Program of the Institute:
Elucidation of the enzymatic processes of activation and mechanisms of action of carcinogens present in the human environment will allow steps to be taken to inhibit formation of active metabolites, to induce detoxifying enzymes, or to provide receptor substances that prevent binding of the active species to cellular macromolecules. Such inhibitors and inducers would be candidate anticarcinogens.

Proposed Course: To continue investigations on:

1. The nature of urinary and microsomal metabolites of the six designated carcinogens.

2. The enzymes involved in carcinogen metabolism and activation.

3. The mechanism of binding of activated metabolites to macromolecules.

The contract will end June 29, 1981.

Date Contract Initiated: June 30, 1975

Current Annual Level: 0

SUMMARY REPORT
CHEMOPREVENTION PROGRAM

Strategies for cancer prevention involving reduction or elimination of human exposure to environmental carcinogens may not always be possible. Further, significant portions of the human cancer burden may be due to endogenous carcinogens, cocarcinogens and promoters. The biological and chemical approach to cancer prevention seeks to inhibit, reverse, arrest or delay the carcinogenic process by administration of selected chemical compounds or combinations of chemical compounds, biological agents or combinations of biological agents, or by combined use of both biological and chemical agents. A large number of studies on experimental animals have already demonstrated inhibition of tumorigenesis at many organ sites such as liver and lung, large and small intestine, breast, skin, bladder, and forestomach. Inhibition has been shown for both chemically-induced and radiation-induced cancers, as well as inhibition of the so-called spontaneously-arising tumors. Furthermore, chemopreventive agents have suppressed malignant and phenotypic transformation in culture and inhibited both UV-and carcinogen-induced bacterial mutagenesis.

Retinoid Program: A number of efforts investigate the chemoprevention of carcinogenesis by retinoids. These natural and synthetic analogs of vitamin A have already been shown to inhibit or delay the development of invasive malignancy in animal models of epithelial carcinogenesis in skin, lung, breast, and bladder, and to suppress malignant and phenotypic transformation in culture whether caused by chemical carcinogens, ionizing radiation, or polypeptide transforming factors derived from virally transformed cells. Effective chemoprevention of epithelial cancer by retinoids will require compounds capable of arresting, reversing, or otherwise inhibiting the carcinogenic process in the desired target organ and tissue, while at the same time, providing sufficiently non-toxic effects upon the host.

For this purpose, several contracts are devoted to the synthesis of new retinoid structures, to assay in vitro for their possible chemopreventive efficacy, to determination of their toxicologic properties and to evaluation of their anticarcinogenic activity in animal systems in vivo. These efforts are providing increased knowledge of retinoid pharmacokinetics and structure-activity relationships.

In the area of retinoid synthesis, three new contracts have been initiated this year. At the University of California (NO1-CP-05717) early emphasis will be placed upon synthesis of the 9,10- and 11,12- allenic retinoids, including several geometric isomers, and their vinylallene alcohols, aldehydes, carboxylic acids and other derivatives. Optimization of synthesis and isolation procedures for intermediates and for coupling reactions have already begun. At Cornell University (NO1-CP-05716) efforts are directed at the synthesis of bicyclic and tricyclic analogues of retinoic acid. The contract will investigate the hypothesis that the precise conformation of the retinoid polyolefin chain may play a role in determining the mechanism and specificity of its action, and that structure-activity relationships involving the flexible side chain of the retinoid can be derived. In the new contract at SRI International (NO1-CP-05600), studies are aimed at the synthesis of analogs having more desirable pharmacologic properties, with both

steric and electronic modifications in the side chain and polar terminus of the retinoid molecule. Structures in both categories have already been synthesized with in vitro biological evaluation completed or underway. Bioassay in hamster tracheal organ culture of the retinoids synthesized in these investigations is performed under a new contract established this year at the IIT Research Institute (NO1-CP-05610, see Contract Narrative under Chemical Research Resources).

A major undertaking in the Chemoprevention Program is the study of the efficacy of retinoids to inhibit or delay the development of cancer in well-defined animal models of carcinogenesis. Retinoids are already known to inhibit or delay carcinogenesis in skin, breast, bladder and respiratory tract. Several important new developments in this area have occurred during the past year. At the Dartmouth Medical School (NO1-CP-85675) studies are in progress on the inhibition of pancreatic carcinogenesis by several retinoids. Although there has been some concern for toxicity in these initial investigations, results to date support an inhibitory role for retinoids against the progression of azaserine-induced neoplasia. These results are important since they indicate that neoplasia at still another organ site can be suppressed by retinoid administration. It is interesting to note that other recent data from this same group demonstrate that azaserine-induced pancreatic carcinogenesis can be both suppressed and enhanced by administration of various semipurified diets.

Three new contracts were initiated this year for investigations on the inhibition of mammary carcinogenesis by retinoids. They reflect a significant broadening of the scope of the retinoid program. New types of efforts at Michigan State University (NO1-CP-05717) will explore combination chemoprevention of dimethylbenz(a)anthracene (DMBA)-induced mammary carcinogenesis in the rat by concurrent administration of retinoids with prolactin secretion inhibitors, with substances known to stimulate the immune system, and with concurrent administration of all three agents. This contract will also investigate the potential of retinoids for inhibition of mammary gland carcinogenesis in three different mouse tumorigenesis models, each possessing certain significant features of human breast cancer: DMBA-induced ductal carcinoma of the mammary gland in C57BL xDBA/2fF$_1$, mice; steroid hormone-induced ductal carcinoma in the GR strain of mouse; and spontaneous mammary carcinomas of alveolar origin in C3H/He mice carrying overt mammary tumor virus. These investigations emphasize the possibility of additive or synergistic effects in combined chemoprevention.

The new contract at the IIT Research Institute (NO1-CP-05718, formerly NO1-CP-75939) will continue chemoprevention efforts in the 1-methyl-1-nitrosourea (MNU) rat model for mammary carcinogenesis employing retinoids alone and in combination chemoprevention studies. Special efforts will include hormonal aspects of chemoprevention. This contractor has found a whole series of retinoids to be effective in delaying the appearance and in reducing the number of mammary cancers; while also demonstrating other retinoids to be ineffective. It is interesting that some of the ineffective retinoids have shown some efficacy in prolonging latency or reducing incidence at other organ sites against other carcinogens. In a recent collaborative study with Dr. Welsch at Michigan State University, this contractor demonstrated that combination chemoprevention of MNU-induced mammary carcinogenesis with retinyl acetate and the prolactin secretion inhibitor 2-bromo-α-ergocryptine was more effective than either treatment alone. Additional combination chemoprevention studies in which administration of the retinoid 4-hydroxyphenyl-

retinamide is combined with ovariectomy have now demonstrated that MNU-induced mammary cancer can again be nearly completely prevented. These studies not only highlight the extremely important new area of combination chemoprevention, but also indicate in an important new finding that retinoids appear to be most effective against hormone independent tumors. The ovariectomy results also indicate that a majority of the MNU-induced tumors are ovarian hormone dependent. Further, in a "lumpectomy" experiment, retinyl acetate (RAc) has been shown to delay the rate of second tumor appearance after the surgical excision of the first tumor; ovariectomy or ovariectomy plus RAc following first excision also very significantly decrease tumor incidence and multiplicity.

At the Brookhaven National Laboratory (YO1-CP-00202) a third new project will investigate the effect of retinyl acetate on X-ray-induced mammary gland carcinogenesis. This project has special significance since radiation is the only known carcinogen for human breast cancer on the one hand, while investigations on retinoid suppression of experimental radiation carcinogenesis have not yet been done on the other.

At the Middlesex Hospital Medical School (NO1-CP-05602, formerly NO1-CP-75938) a new series of efforts has begun on retinoid suppression of N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN)-induced urinary bladder carcinogenesis. In these experiments a newly-developed mouse model (IIT Research Institute, NO1-CP-23292) will be employed in which BBN induces highly invasive transitional cell carcinomas similar to those frequently seen in man. Another study will investigate, in the Fischer rat model, the interesting possibility that retinoids may act as antipromotion agents in BBN-initiated, saccharin-promoted bladder carcinogenesis. This contractor recently completed a detailed series of studies on the effects in F344 rats of life-time administration of two retinoids (13-cis-retinoic acid, CRA, and N-ethyl-retinamide, NER), and the effects these retinoids have on the course of development of bladder cancer. It was shown that the anti-carcinogenic activities of these retinoids could be entirely accounted for by an increase in the latent period before tumor growth commenced from areas of BBN-induced hyperplasia. Neither of these retinoids prevented the development of bladder cancer, nor did they permanently suppress the development of squamous metaplasia or markers of neoplastic transformation, such as pleiomorphic microvilli and changes at the urothelial-mesenchymal interface.

Natural Inhibitors of Carcinogenesis. Three contracts have a special signficance in this program devoted to the chemical modulation of the carcinogenic process. Their objectives are to determine if naturally-occurring materials have the capacity to inhibit the occurrence of neoplasia and to identify such inhibitors. The contract at the American Health Foundation (NO1-CP-85659) assesses the protective role of the dietary fibers, wheat bran and citrus pulp on chemically-induced digestive tract cancer. Last year it was reported that both of these fibers at a level of 15% in the diet are capable of causing a significant reduction in azoxymethane-induced tumorigenesis in the colon and in the small intestine. In these studies, animals were placed on the diets prior to carcinogen administration, and inhibition was demonstrated in both tumor incidence and multiplicity. These results have now been extended to inhibition of 3,2',-dimethyl-4-aminobiphenyl-induced intestinal tract tumorigenesis. Wheat bran again reduced the incidence of tumors and their multiplicity in the colon and small intestine, while dehydrated citrus pulp reduced incidence and multiplicity in the small intestine.

Identification of naturally-occurring inhibitors of neoplasia constitutes an important set of endeavors, not only for the development of chemopreventive agents but also for the critical information such investigations would provide in the evaluation of epidemiological findings of differences in cancer incidence in various population groups. In this regard, studies have been carried out at the University of Minnesota (NO1-CP-85613) on the effect of various varieties of cabbage and cauliflower on benzo(a)pyrene-induced neoplasia of the lungs and forestomach of mice. As reported last year, several interesting results have been obtained. First, significant inhibition of pulmonary adenoma formation by certain varieties of cabbage and cauliflower has indeed been found. Secondly, the time relation between feeding of the vegetables and carcinogen administration is apparently very important in determining whether or not inhibition is found: inhibition of pulmonary adenoma formation occurs when mice are pre-fed the two types of vegetables but not when three varieties of cabbage are fed after carcinogen administration. Further, inhibition of forestomach tumor formation is not found under either feeding protocol. These results suggest that at least certain varieties of cabbage and cauliflower have the capacity to enhance systemic detoxification of B(a)P. These results have now been extended to studies on inhibition of 1,2-dimethylhydrazine-induced colonic neoplasia. Dehydrated, powdered cabbage, broccoli, Brussels sprouts and celery at 20% of the diet were all capable of significantly reducing both incidence and multiplicity of neoplasms in the large bowel when fed prior to and simultaneously with exposures to DMH. Chieftain Savoy cabbage and broccoli were particularly efficacious, each reducing incidence to 23% of the control. Chieftain Savoy cabbage fed subsequent to DMH administration (as the leaf) reduced incidence to 58%, while Brussels sprouts under these conditions at 10% of the diet actually doubled the incidence of large bowel neoplasia. A possible basis for some of the inhibition observed at these organ sites is the inducing capacity which many of these vegetables possess for the glutathione-S-transferase system. This system is one of the major cellular detoxifying elements for many chemical carcinogens.

A new endeavor in this contract (University of Minnesota, NO1-CP-85613) this past year has yielded exciting results on new sources of natural inhibitors. It has been found that green coffee beans from five sources in Central and South America contain a constituent that markedly induces glutathione-S-transferase (GST) activity in both liver and small bowel of the mouse. This constituent is not caffeine, although caffeine does induce GST to a lesser degree. Roasted coffee, instant coffee and instant decaffeinated coffee all have inducing activity. Isolation efforts show that the active constituent is soluble in nonpolar organic solvents, and purified fractions (not containing caffeine) have been obtained using preparative high pressure liquid chromatography. Further, powdered green coffee beans from Guatemala and powdered black tea leaves from Ceylon at 10% of the diet inhibit both the incidence and multiplicity of DMBA-induced mammary carcinogenesis in the rat.

In the contract at Oregon State University (NO1-CP-85660), the well-characterized aflatoxin B_1 (AFB_1)-induced hepatocarcinogenesis model in rainbow trout is being employed to investigate natural inhibitors (and purified chemicals from natural inhibitors) of the carcinogenic process. Results indicate that broccoli, cauliflower, Brussels sprouts and benzylisothiocyanate induce only marginal changes in cytochrome P450, benzo(a)pyrene monooxygenase, ethoxycoumarin deethylase, ethoxyresorufin deethylase and aflatoxicol dehydrogenase. These substances have, so far, failed to alter aflatoxin-induced liver cancer in trout.

Phenolic Antioxidants: The phenolic antioxidants butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are perhaps the most broadly studied of all presently known chemopreventive agents. They are known to effectively inhibit neoplasia induced by many classes of chemical carcinogen at many organ sites. For example, these two compounds are inhibitors of tumorigenesis induced by nitrosamines; polycyclic aromatic hydrocarbons; 4-nitroquinoline-N-oxide; 1,2-dimethylhydrazine; azoxymethane; 4-dimethylaminoazobenzene; N-2-fluorenylacetamide; N-hydroxy-N-2-fluorenylacetamide (N-OH-FAA); uracil mustard; and urethane. Inhibition of neoplasia has been demonstrated for mouse lung, forestomach and skin as well as for rat liver, breast and colon. Moreover, in very recent work, BHA has been shown (a) to inhibit neoplasia of the forestomach, lung and lymphoid tissues of the mouse induced by (±)-trans-7,8-dihydroxy-7,8-dihydrobenzo(a)pyrene, which is considered at the present time to be a proximate carcinogenic metabolite of the parent hydrocarbon; (b) to inhibit mutagenesis induced by known carcinogens and a number of antischistosomacidal compounds; and (c) to inhibit, very effectively, large bowel carcinogenesis induced in mice by methylazoxymethanol acetate.

However, much of this information on inhibition of carcinogenesis by these compounds is available only at high concentrations. For this reason, basic dose-response studies have been initiated this year in four new contracts to investigate BHA and BHT inhibition of neoplasia at four organ sites: mammary gland, liver, colon and lung (American Health Foundation, NO1-CP-05722; NO1-CP-05723; NO1-CP-05721; and University of Minnesota, NO1-CP-05605). Since these agents are already in human use (as food preservatives, for example) knowledge of their current effects and future potential at lower doses is extremely important.

CHEMOPREVENTION

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AMERICAN HEALTH FOUNDATION (NO1-CP-05721)

Title: Dose Response Studies on Phenolic Antioxidants (Intestinal Tract)

Contractor's Project Director: Dr. Bandaru S. Reddy

Project Officer (NCI): Dr. Carl E. Smith

Objectives: The overall objective of the program is to evaluate the efficacy of phenolic antioxidants, namely butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) as chemopreventive agents in intestinal carcinogenesis. Specifically, the program will assess the efficacy of 6000, 3000, 1000 and 300 ppm of dietary BHT or BHA on azoxymethane (AOM)- or methylazoxymethanol (MAM) acetate-induced intestinal carcinogenesis in F344 rats or CF-1 mice, respectively.

Major Findings: The first phase of the program has been initiated to investigate the inhibitory effect of four levels of BHT (6000, 3000, 1000 and 300 ppm) added to NIH-07 diet and one level (6000 ppm) incorporated into semipurified diet on AOM (2 dose levels: 29.6 or 14.8 mg/kg body wt, administered once) - induced intestinal carcinogenesis. BHT containing diets will be fed to male F344 rats 2 weeks before, during and 2 weeks after carcinogen treatment. Diets containing BHA or BHT at 6000, 3000, 1000 and 300 ppm have been analyzed for these antioxidants for quality control purposes so as to assure homogenous distribution and stability of these compounds in the experimental diets. The mixing of these antioxidants in NIH-07 and semipurified diet was performed using a Patterson-Kelly V blender after premixing in small quantities in a food mixer. Aliquots of samples obtained from three different areas of the blender were extracted with CS_2 and analyzed quantitatively for BHA or BHT by GLC (A.O.A.C. methods) using di-BHA as an internal standard. Recoveries from diets containing 300-6000 ppm of BHA or BHT ranged from 88 to 98%. The mean recovery was 94% for BHA and 92% for BHT. These results thus indicate that the method of mixing of antioxidant-containing diets is dependable and adequate for the program.

Significance to Biomedical Research and the Program of the Institute: Chemoprevention which focuses on the inhibition of carcinogenesis by chemical agents is a concept that exists for preventing cancer, not only because these chemical agents prevent carcinogens from reaching or reacting with critical target sites, but they can also inhibit the promotional phase of neoplasia. These chemical agents include, besides other compounds, phenolic antioxidants, such as BHA or BHT. However, in terms of human consumption, there should be detailed dose-response studies on BHA and BHT under defined protocols in animal models. Thus, a data base on the potency of these inhibitors obtained from this program could provide convincing evidence on the inhibitory effect of these antioxidants in colon carcinogenesis. An understanding of the mechanism of the effect of these antioxidants in relation to colon cancer inhibition may provide a sound basis for a reduction of the risk of developing cancer of the colon.

Proposed Course: The effect of dietary BHT at 6000, 3000, 1000 and 300 ppm levels on AOM-induced colon carcinogenesis in male F344 rats will be investigated. In these experiments, BHT will be fed beginning two weeks prior to carcinogen administration and continuing for the entire course of the experiment.

Date Contract Initiated: September 30, 1980

Current Annual Level: $97,482

AMERICAN HEALTH FOUNDATION (NO1-CP-05722)

Title: Dose Response Studies on Phenolic Antioxidants (Mammary Gland)

Contractor's Project Director: Dr. Leonard A. Cohen

Project Officer (NCI): Dr. Carl E. Smith

Objectives: To evaluate, in animal model systems, the efficacy of two phenolic antioxidants, butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), as chemoprophylactic agents in breast carcinogenesis.

Major Findings: The first phase of this project concerned Quality Control and Randomization, which resulted in the following observations:

1. Dimethylbenz(a)anthracene (DMBA), the carcinogen to be used, was obtained from a commercial supplier and analyzed by high performance liquid chromatography (HPLC) and mass spectroscopy to determine purity. Analysis was carried out according to the method of Chou & Yang (J. Chromatog. 185, 635-654, 1979). A single sharp peak eluted at the expected retention time; polar metabolites were not detected. Mass spectral analysis was performed on the DMBA eluted from the column. The major mass ions were in agreement with assigned structures.

2. Diets containing BHA or BHT at 6000, 3000, 1000 and 300 ppm have been analyzed for these antioxidants for quality control purposes so as to assure homogeneous distribution and stability of these compounds in the experimental diets. The mixing of these antioxidants in NIH-07 and semipurified diets was performed using a Patterson-Kelly V blender after pre-mixing in small quantities in a food mixer. Aliquots of samples obtained from three different areas of the blender were extracted with CS_2 and analyzed quantitatively for BHA or BHT by GLC (A.O.A.C. Methods) using di-BHA as an internal standard. Recoveries from diets containing 300-6000 ppm of BHA or BHT ranged from 88 to 98%. The mean recovery was 94% for BHA and 92% for BHT. These results indicate that the method of mixing of antioxidant-containing diets is dependable and adequate for the program.

3. Ten animals were taken at random from the population and subjected to routine screening procedures (histopathology, serology, parasitology) to assess general health of the population.

4. On March 26, 1981, animals were taken from quarantine and randomized into treatment groups before administration of experimental diets. Randomization consisted of sorting into 3-weight classes and assigning cages by use of a random number table. Animals at extreme ends of the weight range were culled from the population prior to randomization.

Significance to Biomedical Research and the Program of the Institute: Phenolic antioxidants such as BHA/BHT are food additives commonly used in the United States to preserve foods containing lipids and particularly polyunsaturated fats. The health consequences to man of consumption of BHA or BHT are unknown at present. Since animal studies indicate that BHA/BHT can inhibit the induction of mammary cancer by specific carcinogens, the possibility exists that these agents may exert a chemoprophylactic effect on the genesis of breast cancer in man. The significance of this study lies in the fact that it permits a quantitative assessment of the effect of BHA/BHT on the development of mammary cancer.

Proposed Course: DMBA will be administered to all treatment groups on April 9, 1981. Experimental diets will be initiated 10 days prior to carcinogen administration and continued for a period of 30 days. Appearance of mammary tumors is expected within 4 weeks of DMBA administration, and this first set of experiments will be terminated in late August, 1981.

Date Contract Initiated: September 30, 1980

Current Annual Level: $102,064

AMERICAN HEALTH FOUNDATION (NO1-CP-05723)

Title: Dose Response Studies on Phenolic Antioxidants (Liver)

Contractor's Project Director: Dr. Gary M. Williams

Project Officer (NCI): Dr. Carl E. Smith

Objectives: The overall objective of this proposal is to evaluate the efficacy of a range of dose levels of the phenolic antioxidant, butylated hydroxytoluene (BHT), as a chemopreventive agent in liver carcinogenesis in rats.

Major Findings: The first phase of the program, involving quality control studies on the preparation of diets containing butylated hydroxytoluene (BHT) at 6000, 3000, 1000 and 300 parts per million has been completed. The mixing of BHT in NIH-07 and semipurified diets was performed using a Patterson-Kelly V blender after pre-mixing in small quantities in a food mixer. Aliquots of samples obtained from three different areas of the blender were extracted with carbon disulfide and analyzed for BHT by gas liquid chromatography. The mean recovery of BHT was 92% with a range of 88 to 98%. These results indicate that the method of mixing of the BHT diet is adequate for the program.

The protocols for study of the effect of BHT on liver carcinogenesis by 2-acetylaminofluorene have been reviewed by the professional staff involved in the program, and the rats for the first year study have been ordered.

Significance to Biomedical Research and the Program of the Institute: Phenolic antioxidants such as BHT are food additives commonly used in the United States to preserve foods containing lipids and particularly, polyunsaturated fats. The health consequences to humans of consumption of BHT are unknown at present. Since animal studies indicate that BHT can inhibit the induction of liver cancer by 2-acetylaminofluorene, the likelihood exists that this agent may exert a chemoprophylactic effect on the genesis of cancer in humans. This study meshes closely with others on the effect of BHT on mammary and colon carcinogenesis.

Proposed Course: In the first series of studies, rats will be exposed to a constant level of 0.02% 2-acetylaminofluorene in the presence of concentrations of BHT ranging from 0 to 6000 parts per million. The inhibitory effect of BHT on the development of preneoplastic liver lesions and liver cancer will be quantified.

Date Contract Initiated: September 30, 1980

Current Annual Level: $85,335

AMERICAN HEALTH FOUNDATION: (NO1-CP-75940)

Title: Long-term Studies on Prevention of Epithelial Cancer by Retinoids

Contractor's Project Directors: Dr. John H. Weisburger
Dr. Jerald Silverman

Project Officer (NCI): Dr. Carl E. Smith

Objectives: To determine the efficacy of certain retinoids in the inhibition of the development of chemically-induced colon cancer of rats during its early stages.

Major Findings: Colon cancer was induced in male F344 rats by intrarectal administration of 2.5mg of methylnitrosourea (MNU) at day 50 and 57 of age. Retinoids, retinyl palmitate (RP) or N-(4-hydroxyphenyl)-all-trans-retinamide (RAHA) were administered intrarectally three times a week at a concentration of 0.03 millimoles. Visualization of developing lesions was accomplished by endoscopy. First tumors were seen, in all groups, nineteen weeks post-induction.

It was found that when RP was administered pre-induction (36 days of age) there was a significantly greater incidence of endoscopically visualized tumors as compared to the MNU control. Significant differences in total tumor incidence, compared to the control, were not seen in the RP group begun post-induction (60 days of age) or when RAHA was begun prior to or after induction. Analysis also indicated that there was a significantly greater number of tumors per rat in the RP pre-induction group than in the control group whereas all other group differences were nonsignificant. For tumor bearing rats there were no differences in the number of tumors per tumor bearing rat as compared to the control in any groups.

A histopathological examination of all treatments, except for RAHA post-induction, showed a significant increase in the average number of tumors per animal; and all treatments significantly increased the number of tumors per tumor-bearing animal compared to the MNU only group. There were no significant differences in the number of malignant tumors between the control and any experimental group. Previous

observations using orally administered retinoids also indicated a lack of significant effect on the inhibition of early development of induced colon cancer.

Significance to Biomedical Research and the Program of the Institute: A major goal in cancer prevention is to modify the course of carcinogenesis in its early promotional phase, or if possible, to prevent initiation and/or promotion. In a number of epithelial organ systems, retinoids have demonstrated a protective effect in reducing the incidence and the number of tumors per animal. The aim of this program is to provide a research base for chemoprevention of a major human cancer; that of a large bowel.

Proposed Course: Contract terminated April 30, 1981.

Date Contract Initiated: September 30, 1977

Current Annual Level: 0

AMERICAN HEALTH FOUNDATION (NO1-CP-85659)

Title: Studies of Natural Inhibitors of Chemical Carcinogens

Contractor's Project Director: Dr. Bandaru S. Reddy

Project Officer (NCI): Dr. Carl E. Smith

Objectives: Epidemiologic studies indicate that the occurrence of colon cancer is associated with a high dietary intake of fat and with a lack of dietary fiber. The overall objective of this program is to identify naturally occurring fibers suitable for addition to normal diets in order to inhibit the development of colon cancer. Specifically, the program will assess the protective role of various dietary fibers, namely, wheat bran (cereal-based) and dehydrated citrus pulp (fruit-based) on azoxymethane (AOM)- or 3,2'-dimethyl-4-aminobiphenyl (DMAB)- induced colon carcinogenesis in F344 male rats, and identify the individual components of fibers that exhibit a protective effect in colon carcinogenesis.

Major Findings: Weanling male inbred F344 rats were obtained commercially at 40 days of age. All animals were allotted at random into experimental groups and fed, ad libitum, one of the semipurified diets containing 0 or 15% wheat bran or dehydrated citrus pulp. At 7 weeks of age, all animals, except vehicle-treated animals, received weekly s.c. injections of 50 mg DMAB/kg body wt for 20 weeks and autopsied 20 weeks later. The animals fed the wheat bran and treated with DMAB had a lower incidence (number of animals with tumors) and multiplicity (number of tumors/tumor-bearing animal) of colon and small intestinal tumors than did those fed the control diet and treated with DMAB. Animals fed the diet containing citrus pulp developed fewer small intestinal tumors (incidence and multiplicity) than did those on the control diet; the number of adenocarcinomas was reduced in rats fed the citrus pulp diet. These results, and those reported in 1980 using AOM as a carcinogen, indicate that diets containing wheat bran and citrus pulp reduce the risk for DMAB- or AOM-induced intestinal cancer and that, in addition, dietary wheat bran inhibit DMAB- or AOM-induced colon carcinogenesis. Thus, the protection against colon cancer depends on the type of fiber and nature of carcinogen.

The activities of bacterial β-glucuronidase, 7α-dehydroxylase, cholesterol dehydrogenase and 7α-hydroxysteroid dehyrogenase in the colonic contents and intestinal mucosa (mammalian) β-glucuronidase activity were determined in vehicle treated rats fed various diets. Animals fed the wheat bran or citrus pulp had lower β-glucuronidase activity in the colonic musosa than did rats fed the wheat bran or citrus pulp. No significant difference was observed in small intestinal mucosal β-glucuronidase activity among the different groups. Bacterial β-glucuronidase and 7α-hydroxysteroid dehydrogenase activities were lower in animals fed the wheat bran than they were in rats fed the control diet. No significant differences were noted in bacterial 7α-dehydroxylase and cholesterol dehydrogenase activities among the dietary groups. These results suggest that the dietary wheat bran or citrus pulp modify certain bacterial and intestinal mucosal enzymes.

Significance to Biomedical Research and the Program of the Institute: This program is of special significance since it is designed to provide important information on the relationship between dietary fibers and their components and colon carcinogenesis. It is hoped that the data base generated in animal models can enhance significantly our knowledge of the controlable etiologic factors which play a role in cancer of the large bowel. The long-term goal is to provide a basis for rational prevention by dietary means of a disease affecting over 100,000 individuals per year in the United States.

Proposed Course:
1. Determine the effect of feeding wheat bran and citrus pulp on the fecal bile acid profile.

2. Experiments are in progress to test the protective effect of hemicellulose (using corn bran as a source) in AOM-induced colon carcinogenesis and are now in their 20th week of post-carcinogen treatment.

Date Contract Initiated: September 30, 1978

Current Annual Level: 0

BATTELLE COLUMBUS LABORATORIES (NO1-CP-85650)

Title: Studies on the Toxicology of Retinoids

Contractor's Project Director: Dr. Perry J. Kurtz

Project Officer (NCI): Dr. Carl E. Smith

Objectives: Conduct short-term toxicity studies in laboratory rats and mice in order to evaluate the relative toxicity of synthetic retinoid compounds. The results are expected to assist in the selection of retinoid compounds for use in human cancer chemoprevention.

Major Findings: During the past year, toxicological studies with three synthetic retinoids have been performed. These studies in rats consisted of a 91-day study with N-(2-carboxyphenyl) retinamide, and 21-day studies with 5-tetrazoyl-all-trans-retinamide and 5-tetrazoyl-13-cis-retinamide.

In the 91-day study with N-(2-carboxyphenyl) retinamide, none of the characteristic indicators of retinoid intoxication (i.e., anemia, long-bone osteoporesis, and histopathological change) were present, nor were there any additional indications of toxicity. These findings were in contrast to earlier studies using equivalent dosages of a close structural analog, N-(4-carboxyphenyl) retinamide. In the latter study, all three major characteristics of retinoid intoxication were observed.

In 21-day preliminary studies with 5-tetrazoyl-13-cis-retinamide and 5-tetrazoyl-all trans retinamide, the latter compound (all-trans) produced evidence of retinoid intoxication at a dose which provide no observable effects in the former (13-cis). Specifically, long-bone osteoporesis with fracturing was observed radiographically in both sexes receiving the high dose of 5-tetrazoyl-all trans retinamide. All other parameters evaluated for both chemical were similar to control values.

All three synthetic retinoids have been shown to be substantially less toxic than the naturally occurring forms.

Significance to Biomedical Research and the Program of the Institute: Based on the toxicity observed throughout the program, N-(2-carboxyphenyl) retinamide appears to be a more attractive candidate for further development as a chemopreventive agent than N-(4-carboxyphenyl) retinamide.

Proposed Course: Five new synthetic retinoids are now scheduled for evaluation in rats and mice which will provide additional data concerning the comparative toxicity of retinoid analogs.

Date Contract Initiated: September 30, 1978

Current Annual Level: $271,406

CALIFORNIA, UNIVERSITY OF (NO1-CP-05715)

Title: Synthesis of New Retinoids for the Chemoprevention of Epithelial Cancer

Contractor's Project Director: Dr. William H. Okamura

Project Officer (NCI): Dr. Carl E. Smith

Objectives: The objectives of the four-year program which began on August 30, 1980, are outlined below. As proposed in the contract, Phase one of the research is projected as a two-year program in which it is planned to complete Items 1 and 2 and begin Item 3 listed below:

1. Synthesize 9,10-allenic retinoids including the (7E,12Z), (7E,12E), (7Z,12Z) and (7Z,12E) geometric isomers; the vinylallene alcohols, aldehydes, carboxylic acids, esters, etc., of these allenic retinoids are target compounds of interest.

2. Similarly, synthesize 11,12-allenic retinoids including the (7E,9E), (7E,9Z), (7E,9E) and (7Z,9Z) geometric isomers. As in item 1, synthesis of the carboxylic acids and other derivatives are target compounds of interest.

3. Perform studies on thermal isomerization and base catalyzed rearrangements of the compounds in items 1 and 2 as routes to additional retinoids for bioassay. As parts of these studies, synthesis will be pursued of alkylated and ring-fused analogues.

4. Similar to the efforts in Items 1, 2 and 3, above, perform synthesis of 7,8-allenic retinoids and their rearrangement reactions.

5. Synthesize acetylenic retinoids in which the double bonds of the natural molecules are replaced by triple bonds.

6. Synthesize 6,7- and 10,11-allenes after the biological potential of the allenic retinoids and isomerization products above have been determined.

Major Findings: This report covers the results obtained during the first five months of the contract project, which was begun on August 30, 1980.

The (Z)-and (E)-geometric isomers of 1-bromo-2-methyl-4-(t-butyldimethylsiloxy) butene were synthesized in 20-40 gram quantities starting from isopentenyl alcohol in three steps. The key operation was the optimization of preparative high pressure liquid chromatography conditions (Waters Prep-500 system) for separating the (Z)- and (E)-bromo alcohols. These two components are the five carbon fragments which correspond to the terminus of the side chain of the target 9,10-allenic retinoids. The results constitute a significant improvement for obtaining truly preparative amounts of these key materials.

The synthesis of 9-ethynyl-β-ionolbenzoate in quantity by our previously described method starting from β-ionone in two steps has offered no problems. The benzoate corresponds to the remaining 15-carbon fragment that makes up the final 20-carbon target retinoid skeleton (the 9,10-allenes). Attempts to prepare the 9-propynyl analog (ultimately leading to 11-methyl retinoids) in a similar way has thus far not proven successful.

With preparative amounts of 5-carbon and 15-carbon synthons needed for preparing the 20-carbon 9,10-allenic retinoids (specifically, (12Z)- and (12E) -10,14-retro-retinol) in hand, more recent studies have been directed towards coupling the 5-and 15-carbon fragments. Conditions for converting the 5-carbon (E)- bromide to the corresponding lithium and mixed cuprate derivatives have not yet been optimized. The corresponding (Z)- bromide appears to be less of a problem. It is anticipated that the two 9,10-allenic retinoid geometric isomers should be available for testing within the next few months.

Significance to Biomedical Research and the Program of the Institute: A significant relationship between the role of vitamin A in controlling differentiation of epithelial cells and the inhibition of development of malignancy in epithelial cells has been established. The natural all-trans form of vitamin A has been reported to have beneficial effects in the prophylaxis of various types of carcinomas, but suffers from excessive localization in the liver leading to toxic liver damage. By contrast, the synthetic analogs, 13-cis-retinoic acid and retinyl methyl ether, are far less toxic but possess similar biological effectiveness in controlling cell differentiation. Thus, the search for an even more effective cancer prophylactic drug in the domain of retinoidal synthetic unnatural products would appear to be both conceptually and practically a worthwhile goal, especially in view of the importance of the cancer problem. It is the purpose of this contract to

systematically investigate the incorporation of reactive allenic and acetylenic functional groups into the retinoid skeleton by analogy with similar useful and interesting studies of other inhibitory biological molecules. Furthermore, these same allenic retinoids will be used in a synthetic sense for rearrangements to new hindered geometric isomers of the normal retinoid skeleton and analogs which otherwise would be difficult to synthesize.

Proposed Course: The proposed course during the next 12 months includes completion of much of Item 1 and portions of Items 2 and 3. It is anticipated that the two 9,10-allenic retinoid geometric isomers, perhaps derivatized as their esters to enhance stability, will be available for testing within the next few months.

Date Contract Initiated: August 30, 1980

Current Annual Level: $69,050

CORNELL UNIVERSITY (NO1-CP-05716)

Title: Synthesis of New Retinoids for the Chemoprevention of Epithelial Cancer

Contractor's Project Director: Dr. John E. McMurry

Project Officer (NCI): Dr. Carl E. Smith

Objectives: Synthesis of bicyclic and tricyclic analogues of retinoic acid as a means of probing the relationship between retinoid activity and side chain conformation.

Major Findings: The initial work under this contract aimed at the synthesis in bulk of some of the major precursors necessary for synthesis of both aromatic and alicyclic analogues of retinoids. This work has not yet produced finished analogues for biological testing. However, the progress of the coming year will take full advantage of the preliminary work.

In addition to the synthesis of a supply of needed starting material, a familiarity with the analytical techniques necessary for the handling and characterization of sensitive new retinoids has been gained. Preparative HPLC, in particular, is now in operation and should prove invaluable.

Significance to Biomedical Research and the Program of the Institute: The present program is aimed both at preparing new retinoid analogues which are more potent, less toxic, and more site specific than known compounds, and at probing structure-activity relationships involving the flexible retinoid side chain. Both goals will increase our understanding of the biology of retinoid action.

Proposed Course: With preliminary preparation of necessary synthetic intermediates now nearing completion, the proposed course of this project is to begin synthesis of a number of bicyclic and tricyclic retinoid analogues in a systematic search for active compounds. The first such compounds should be available in the near future.

Date Contract Initiated: August 30, 1980

Current Annual Level: 0
(multi-year funded FY 80)

DARTMOUTH MEDICAL SCHOOL (NO1-CP-85675)

Title: Prevention of Pancreatic Cancer in Experimental Animals by Retinoids

Contractor's Project Director: Dr. Daniel S. Longnecker

Project Officer (NCI): Dr. Carl E. Smith

Objectives: The primary objective of this project is to test the hypothesis that specific synthetic retinoids which are less toxic than vitamin A can prevent development of, or slow the progression of, epithelial cancers of the pancreas. To accomplish this goal we have proposed to induce carcinomas or preneoplastic epithelial lesions in the pancreas of rats treated with azaserine and hamsters treated with N-nitrosobis(2-oxopropyl)-amine, and then to see if there is a decrease in incidence, size, or number of cancers or preneoplastic lesions among the animals given retinoid-supplemented diets during the latent period of carcinogenesis.

Major Findings: The project is divided into two phases, each of which consists of initiation, feeding, autopsy, and histologic evaluation of the pancreas in groups of carcinogen-treated and control rats and hamsters which were fed diets supplemented with one of four different retinoids or the appropriate vehicle solvent. The first phase of the project has been completed and these will be identified as studies with "group 1 retinoids". Studies with "group 2 retinoids" are in progress. The major portion of this report will summarize the experience with the group 1 retinoids.

The retinoids included in group 1 are N-(2 hydroxyethyl) retinamide, N-(4-propionyloxyphenyl) retinamide, and retinylidene dimedone, all fed to rats at 2.0 or 1.0 mmoles per kg of diet and to hamsters at 1.0 or 0.5 mmoles per kg of diet. The fourth retinoid, N-(4-carboxyphenyl) retinamide, was fed to rats at 1.0 mmole per kg of diet and to hamsters at 0.5 mmole per kg diet. Due to problems with maintaining satisfactory growth rates caused by toxicity, the dietary levels were reduced in several groups from the levels listed above.

Three of the group 1 retinoids appear to have inhibited the progression of azaserine-induced pancreatic neoplasms in Lewis rats. In the retinoid-fed rats, the incidence of carcinomas was decreased in males; the incidence of adenomas, carcinomas-in-situ, and carcinomas (combined) was decreased in females; and the average pancreatic weight was reduced in retinoid treated groups of both sexes. The effective retinoids were retinylidylidene dimedone which was the least toxic, N-(2-hydroxyethyl) retinamide, and N-(4-propionyloxyphenyl) retinamide. The N-(4-carboxyphenyl) retinamide was more toxic and less effective in chemoprevention.

Poor survival of hamsters led to early termination of this experiment at 40 weeks rather than 52 weeks post-treatment with BOP. The combination of small groups and low incidence of adenocarcinomas among females limits the significance of this data even though it consistently shows a reduced incidence of carcinoma among the retinoid-treated groups of both sexes. There was significant reduction of

carcinomas among male hamsters treated with retinylidene dimedone and N-(2-hydroxyethyl) retinamide. It is of interest that these were the two most effective compounds in both rats and hamsters. It may ultimately be desirable to study these compounds again in hamsters using lower, less toxic doses.

Significance to Biomedical Research and the Program of the Institute: The demonstration that synthetic retinoids are effective in inhibiting the progression of experimentally induced pancreatic carcinomas in rats and hamsters indicates that the pancreas can be classed with the breast, bladder and lung in which a similar effect of retinoids has been shown. This observation provides an additional model system in which the mechanism of progression of malignancy can be studied and may ultimately provide a clinical approach for inhibiting the development of pancreatic cancer among selected individuals or groups who are deemed to be at high risk for development of this disease.

Proposed Course: Evaluation of the effectiveness of the group 2 retinoids in chemoprevention of pancreatic cancer in the hamster and rat models is in progress. The group 2 retinoids are N-4-pivaloyloxyphenyl retinamide, N-2-hydroxypropyl retinamide, N-3-hydroxypropyl retinamide, and N-2,3-dihydroxypropyl retinamide. These were initially fed at the levels of 2.0 or 1.0 mmole per kg of diet for rats, and 0.5 and 0.25 mmole per kg of diet for hamsters. The studies with rats were begun in May, 1980 and with hamsters in July, 1980. Thus, the scheduled sacrifice dates fall in June-September, 1981. Autopsy data, histologic study, and evaluation remain to be completed during the last seven months of the project beginning in June, 1981.

Date Contract Initiated: September 30, 1978

Current Annual Level: $124,211

DEPARTMENT OF ENERGY, BROOKHAVEN NATIONAL LABORATORY
(Y01-CP-00202)

Title: Chemoprevention of Epithelial Cancer by Retinoids (Mammary Gland)

Contractor's Project Director: Dr. Claire J. Shellabarger

Project Officer (NCI): Dr. Carl E. Smith

Objectives: To determine if the retinoid inhibition of chemically induced rat mammary carcinogenesis can be extended to x-ray-induced and spontaneous mammary carcinogenesis; to determine if the retinoid-inhibition can be maintained over the rat's entire lifespan for chemically-induced, x-ray-induced, and spontaneous mammary carcinogenesis; and to study the toxicological, physiological, and endocrinological changes produced by long-term retinoid treatment.

Major Findings: Since the present contract limits the long-term study of retinyl acetate to a single dose level (given as a supplement in the food), a small preliminary study was begun to assess the toxic effects of low levels of retinyl acetate in the diet (5mM, 1mM, and 0.5mM/Kg diet) under our test conditions. Early results (35 days after beginning the retinyl acetate supplemented diet) indicate that only the rats receiving 5mmoles retinyl acetate/Kg diet showed significant

weight loss (<40g). This weight loss is an early symptom of retinyl acetate toxicity. During this same period, control rats and rats receiving 0.5 or 1.0mM retinyl acetate/kg diet gained over 22g.

Results are not available from the main experiment, but an initial group of 300 rats is presently being aged prior to being treated with the carcinogens and is being placed on the retinyl acetate supplemented diet.

The high performance liquid chromatographic procedures for analysis of the retinyl acetate in the diet have been worked out, and diet samples from the preliminary experiment have been satisfactorily assayed.

A large twin-shell cross-flow blender (70 kg per batch) has been obtained for mixing the rat chow and retinyl acetate beadlets.

Significance to Biomedical Research and the Program of the Institute: If retinoids are to be used in the chemoprevention of human breast cancer, more information will be required than now exists about their inhibitory action on animal mammary carcinogenesis and their toxicity. In the present contract, mammary carcinogenesis induced by a chemical carcinogen, induced by x-rays, and occurring spontaneously in female Sprague-Dawley rats is used as a model system for human breast cancer. This model system is used because the hormonal control and histopathology of rat and human mammary adenocarcinomas is similar. The current experiments are designed to provide information about the extent to which retinoids can arrest, delay, or reverse mammary carcinogenesis in this established model system.

It has been demonstrated by other investigators that certain retinoids can inhibit chemically-induced mammary carcinogenesis in female rats for periods up to 7 months. The present investigation is designed to determine:

1. If this inhibition can be extended to x-ray induced mammary carcinogenesis.

2. If this inhibition can be maintained over the entire lifespan and can reduce the final adenocarcinoma incidence.

3. If this inhibition can be seen for spontaneously occurring mammary carcinogenesis.

In addition, the toxicological, physiological, and endocrinological changes produced by long-term retinyl acetate treatment will be studied.

Proposed Course: Within the next four months, all the experimental and control rats (500) specified in the contract will be treated with their respective carcinogens and placed on either the retinyl acetate supplemented diet or the control diet. The level of retinyl acetate to be used in the diet will be determined after evaluation of the data accumulated during the present preliminary toxicity studies. Ten animals within each group will be used to monitor toxicological, physiological, and endocrinological changes produced by long-term retinoid treatment of these animals.

During the rest of the contract period, the animals will be maintained and data will be collected and analyzed.

Date Contract Initiated: September 19, 1980

Current Annual Level: $201,862

IIT RESEARCH INSTITUTE (NO1-CP-05718) (Formerly NO1-CP-75939)

Title: Chemoprevention of Epithelial Cancer by Retinoids (Mammary Gland)

Contractor's Project Director: Dr. Richard C. Moon

Project Officer (NCI): Dr. Carl E. Smith

Objectives: The major goal of this project is to assess the chemopreventive activity of retinoids (natural and synthetic analogues of vitamin A) in long-term studies conducted in rodent models for breast cancer.

Major Findings: Previous work has indicated that retinyl acetate, retinyl methyl ether, 4-hydroxphenyl retinamide, 13-cis-4-hydroxyphenyl retinamide and axerophthene significantly prolong the latency of mammary cancer appearance and reduce the number of mammary cancers induced by 1-methyl-1-nitrosourea (MNU). On the other hand, retinyl butyl ether, retinylidene dimedone, retinylidene 2,4-pentadione, 2-hydroxyethyl retinamide and ethyl retinamide were ineffective againts MNU-induced mammary cancer in Sprague-Dawley female rats. In these studies, 4-hydroxyphenyl retinamide was well tolerated. In an ongoing lifetime mammary carcinogenesis study (low dose carcinogen) the chemopreventive activity of 4-hydroxyphenyl retinamide in continuing to be evaluated.

In additional combination chemoprevention studies, the combined effect of 4-hydroxyphenyl retinamide and ovariectomy almost completely prevented MNU-induced mammary cancer and further indicated that retinoid responsive tumors in most cases are hormone independent tumors. The retinoids also delay the rate of second tumor appearance following the surgical excision of the first tumor.

Significance to Biomedical Research and the Program of the Institute: Studies performed under this contract indicate that efforts to synthesize organotrophic retinoids with increased chemopreventive activity and diminished toxicity in comparison to previously tested compounds are meeting with success. The data obtained from long-term evaluation of retinoids will hopefully lead not only to the establishment of the concept of cancer chemoprevention, but will also provide evidence for the use of retinoids in suppressing progression of early neoplastic lesions in women who are at high risk for breast cancer.

Proposed Course: As they become available, newly synthesized retinoids will be evaluated for chemopreventive activity in rodent models for breast cancer.

Date Contract Initiated: September 30, 1980

Current Annual Level: $113,344

IIT RESEARCH INSTITUTE (NO1-CP-75939)

See the new contract award NO1-CP-05718.

MICHIGAN STATE UNIVERSITY (NO1-CP-05717)

Title: Chemoprevention of Epithelial Cancer by Retinoids (Mammary Gland)

Contractor's Project Director: Dr. Clifford W. Welsch

Project Officer (NCI): Dr. Carl E. Smith

Objectives:

1. To enhance the mammary tumorigenic suppressive activities of retinoids in the carcinogen treated Sprague-Dawley rat by concurrent hormone inhibition and/or immune stimulation.

2. To determine whether or not retinoids possess mammary tumorigenic suppressive activities in 3-distinctly different types of mouse mammary carcinoma models, i.e., the carcinogen treated C57BL X DBA/2fF_1 mouse, the hormone treated GR mouse, and the spontaneous (overt MTV) C3H/He mouse.

Major Findings: This research project was begun just 6 months ago. 640 female Sprague-Dawley rats and 300 female C3H/He mice have been purchased and placed in experimentation. The data obtained to date is too preliminary to draw any definitive conclusions.

Significance to Biomedical Research and the Program of the Institute:
The administration of retinoids to laboratory rats shortly after carcinogen treatment suppresses the development of mammary carcinoma, i.e., mammary tumor incidence in retinoid treated rats is reduced by approximately 50%. Thus, retinoid treatment alone in these studies is not capable of totally preventing the emergence of early mammary gland neoplasia in carcinogen treated rats. The therapeutic modalities of immune stimulation or drug induced hormone suppression, by themselves, are also not totally effective in preventing the emergence of these neoplasms. It is believed and hoped to be shown, that a combination of the 3 most promising non-toxic prophylactic modalities (for breast cancerigenesis) available to oncologists today (i.e., retinoid feeding, hormone inhibition, and immune stimulation) will be 100% effective in the chemoprevention of experimental mammary gland carcinogenesis. Enhancement of retinoid activity by the concurrent use of other mammary oncolytic therapeutic modalities has not heretofore been attempted.

An additional very important concern is the realization that to date, the well acknowledged chemopreventive capabilities of retinoid feeding in experimental mammary gland tumorigenesis has only been demonstrated in one rodent model, i.e., the carcinogen induced rat mammary tumor model. With one exception, other rodent mammary tumor models have not been evaluated. Is it possible that the chemopreventive activities of retinoids in murine mammary gland tumorigenesis are

confined to only one murine mammary cancer model? It is very important, therefore, to test the chemopreventive activities of retinoids in a variety of rodent mammary tumor models; rodent models each of which possess significant morphological and physiological characteristics of human breast cancer, not shared by each other.

Proposed Course: The studies of retinoid inhibition of induced and spontaneous mammary gland cancer in rat and mouse systems will continue with emphasis also on combined chemoprevention.

Date Contract Initiated: September 19, 1980

Current Annual Level: $68,523

MIDDLESEX HOSPITAL MEDICAL SCHOOL
(NO1-CP-05602) (Formerly NO1-CP-75938)

Title: Chemoprevention of Epithelial Cancer by Retinoids (Bladder)

Contractor's Project Director: Dr. R. Marian Hicks

Project Officer (NCI): Dr. Carl E. Smith

Objectives:

1. To assess the efficacy of two retinoids, 13-cis retinoic acid (CRA) and N-ethyl-retinamide (NER), in delaying and/or preventing the development of bladder cancer in Female F344 rats given 1,200 mg, 600 mg or 300 mg of the bladder carcinogen butyl-hydroxybutyl nitrosamine (BBN).

2. To assess whether the retinoid therapy produces any toxicity when administered to rats for life (2 yrs.).

Major Findings: Both retinoids were palatable in the diet at the doses administered and were well tolerated over the 2-year test period. However, the significant reduction in body weights of animals fed the higher dose of CRA (480 mg/kg) or either dose of NER (327 mg/kg or 654 mg/kg) observed at 6 months, was apparent until the final kills at 2 years. Furthermore, in animals fed CRA or NER femoral width was significantly reduced due to a decreased width of the marrow cavity, suggesting that the retinoids affect bone remodeling. However, no fractures were observed. Neither retinoid increased or decreased survival in groups not reciving carcinogen, nor were any overt signs of retinoid intoxication, such as those observed in clinical trials, apparent over the 2 year period.

By 2 years, almost all animals given 1,200 or 600 mg of the carcinogen BBN were dead, mainly as a result of their bladder tumors, irrespective of the presence or absence of either CRA or NER in the diet. Life tables constructed for deaths due to bladder tumors, however, demonstrated a shift of the survival curves of retinoid-treated rats by 7-10 weeks along the time axis, reflecting a similar increase in time before deaths due to bladder tumors commenced in these animals. This shift

demonstrated that, at any point in time studied, the estimated chance in survival was significantly greater in the retinoid-fed groups than in those given carcinogen only. A similar shift was apparent in the life tables of rats given 300 mg BBN and NER, when deaths due to all causes were considered.

Following the demonstration at 1 year that significantly smaller bladder tumor volumes were present in animals fed NER after 1,200 or 600 mg BBN, the development of bladder cancer was studied in pairs of carcinogen-treated rats fed CRA or NER, but killed sequentially. Average total bladder tumor volumes were significantly smaller in NER-treated rats at any point in time, up to 84 weeks, when compared to placebo-fed controls. Regression analysis of volumes when logged and of log volume of the largest tumor present in each bladder, versus time, demonstrated that tumor growth was exponential in both placebo-fed and NER-fed rats, but that NER neither accelerated nor decelerated the rate of tumor growth, since the slopes of the fitted regression lines were parallel. However, the line for the NER-fed group was shifted along the time axis, indicating that the retinoid extended that latent period before tumor growth was established by 8-10 weeks.

Histopathological assessment of the bladders of these rats demonstrated the incidence and severity of proliferative lesions to be reduced, and more areas of normally differentiated urothelium to be present at any point in time studied in NER-treated rats. But regression analysis indicated that the rate at which areas of the bladder became involved by proliferative lesions was the same in NER-treated animals as in placebo-fed controls. The decreased percentage incidence and severity of lesions in the NER-treated group could be explained, however, by an increased latent period of 7-10 weeks, before proliferative lesions, including carcinomata, appeared in the NER-treated animals. Ultrastructural studies confirmed that the appearance of markers of neoplastic transformation, such as pleiomorphic microvilli and changes at the urothelial-mesenchymal interface, were directly related to this increased latent period to CRA or NER-treated rats, and were not permanently suppressed.

Thus, neither CRA nor NER prevented development of, or deaths due to, bladder cancer, nor measurably reduced the rate of tumor growth, nor prevented the development of squamous metaplasia or markers of neoplastic transformation. They did, however, prolong the latent period before tumor growth commenced, by 8-10 weeks, and this was reflected in the life tables. Thus, at any point in time a greater proportion of bladder tumors were small and better differentiated in the retinoid-fed groups than in those in placebo-fed controls.

Significance to Biomedical Research and the Program of the Institute:
The results of these experiments confirm previously published reports that, at any point in time, the incidence and severity of bladder pathology in retinoid-fed animals was reduced when compared to placebo-fed controls. In the studies reported here, however, this effect of the retinoids tested appears to be due to a prolongation of the latent period, during which carcinogen-induced pre-neoplastic changes remain quiescent before exponential tumor growth commences, rather than due to a direct effect on growth rate of the tumors, or suppression of any malignant phenotypes.

The ability of retinoid therapy to delay tumor growth in any already neoplastically transformed urothelium holds great potential for the management of patients who have already had one bladder carcinoma resected and who are known to be at risk of developing further urothelial neoplasms. Even a delay in tumor development of as

little as 7 to 10 weeks in the rat could conceivably equate to an extra 5 or 6 years of symptom-free life for the patient, providing he could tolerate the same levels of retinoids as can the rat. However, since both retinoids at the doses used in these studies produce some toxicity even in the rat, it becomes of great importance to test further analogs in the hope of discovering those which can combine a maximum therapeutic efficacy with the minimum of toxicity.

Proposed Course: In the animal model for bladder cancer used in these experiments, carcinogenic treatment terminated before the animals were exposed to retinoids in the diet. The retinoids could not, therefore, have acted as anti-initiators nor as anti-promoters, but only at some later stage to tumor progression. There is already considerable evidence that some retinoids can act as anti-promoters in skin carcinogenesis (eg. Verma, et al., 1979, Cancer Res. 39 419-425; Slaga et al., 1980, Proc. Natl. Acad. Sci. U.S.A. 77, 2251-2254). Therefore, by use of the established in vivo model of BBN-treated F344 rat, it is proposed to initiate carcinogenesis in the urothelium with a low dose of BBN and to investigate the ability of retinoids to antagonise the promoting effect of saccharin. The effect on promotion of a delay (in the use of retinoid) will also be studied.

The bladder tumors produced in the BBN/rat model are frequently exophytic and local invasion of the bladder wall is often only superficial. In order to simulate the situation commonly experienced in man, where bladder carcinoma arising from areas of carcinoma in situ exhibits an agressive, infiltrating growth pattern, a mouse model (C57/Blb x DBA/2) developed in Dr. Moon's laboratory (Thompson, et al., 1981 Cancer Res. in press) will be used in which BBN induces highly invasive transitional cell carcinomata to test further synthetic retinoids.

Date Contract Initiated: September 30, 1980

Current Annual Level: $147,551

MIDDELSEX HOSPITAL MEDICAL SCHOOL (NO1-CP-75938)

See the new contract award NO1-CP-05602.

MINNESOTA, UNIVERSITY OF (NO1-CP-05605)

Title: Dose-Response Studies of Phenolic Antioxidants

Contractor's Project Director: Dr. Lee W. Wattenberg

Project Officer (NCI): Dr. Carl E. Smith

Objectives: The basic objective of this contract is to determine the efficacy of 2(3)-butylated hydroxyanisole (2(3)-BHA) in inhibiting carcinogen-induced pulmonary adenoma formation in the mouse. An important component of this objective is to determine whether low doses of 2(3)-BHA, of the magnitude likely to be consumed by man, inhibit chemical carcinogens, and the conditions under which inhibition would occur.

Major Findings: A series of experiments have been initiated to determine the effects of varying concentrations of 2(3)-BHA on benzo(a)pyrene-induced pulmonary adenoma formation. Thus far, four such experiments have been started using dose levels of BHA varying from 0.33 mg/GM of diet to 5.0 mg/Gm of diet. BP doses employed are 2 mg, 0.7 mg, 0.2 mg and a non-carcinogen control.

The commercial preparation of BHA contains two isomers. The major isomer is 3-tert-butyl-4-hydroxyanisole and the minor isomer is 2-tert-butyl-4-hydroxyanisole. Work is also in progress to determine the ratio of these two isomers which produces a maximum induction of increased glutathione S-transferase activity and increased tissue levels of glutathione. The following ratios of major to minor isomers of BHA have been employed: 10:0, 9:1, 3:1, 1:1, 1:3, 1:9 and 0:10. An experiment has been completed in which a dietary concentration of 1 mg/Gm of diet and a feeding period of four weeks were employed. In liver, high inducing effects were obtained with both parameters at ratios of major to minor isomer of one or higher. The same pattern was also found for the mucosa of the small intestine. In contrast, the mucosa of the forestomach showed a different pattern. In this tissue, the inductive effects of the minor isomer of BHA is considerably greater than the major isomer. Maximum levels of induction occur at ratios of major to minor isomer of one or less.

Significance to Biomedical Research and the Program of the Institute: 2(3)-BHA is an antioxidant widely used as a food additive. 2(3)-BHA has been found to inhibit a diverse group of chemical carcinogens. An elucidation of its capacity to inhibit at low concentrations would give information as to its potential impact as an inhibitor of environmental carcinogens to which humans are exposed. A knowledge of the optimal isomer concentrations for obtaining inhibition would be of importance in decisions as to its most effective use.

Proposed Course: The course as outlined in the original contract will be followed.

Date Contract Initiated: September 30, 1980

Current Annual Level: $120,518

MINNESOTA, UNIVERSITY OF (NO1-CP-85613)

Title: Studies of Natural Inhibitors of Chemical Carcinogens

Contractor's Project Director: Dr. Lee W. Wattenberg

Project Officer (NCI): Dr. Carl E. Smith

Objectives: The objectives of this project are to determine if naturally-occurring materials have the capacity to inhibit the occurrence of neoplasia and to identify such inhibitors.

Major Findings:
1. Studies of the effects of diets containing vegetables on carcinogen induced neoplasia of the large intestine in CF^1 mice. Two sets of experiments have been carried out to determine the effects of vegetables on carcinogen-induced neoplasia of the large intestine. In the first, the vegetables were administered prior to and simultaneously with exposure to the carcinogen, and in the second, the vegetables

were administered subsequent to carcinogen exposure. Female CF[1] mice were randomized by weight when they were eight weeks of age. They were placed on diets containing dehydrated, powdered vegetables or a corresponding control diet. The experimental diets were fed for two weeks prior to the initial dose of 1,2-dimethylhydrazine (DMH) and continued until one day after the last dose. DMH 0.6 mg was given subcutaneously two times a week for eight weeks. The experiment was terminated 52 weeks after the intial dose of DMH. The results were as follows (ratio of % of mice fed the vegetable diet showing neoplasia of the large bowel/controls): Chieftain Savoy cabbage 0.23, broccoli 0.23, Brussels sprouts 0.46, and celery 0.61. Thus, all of the vegetables exerted an inhibitory effect; the most potent being Chieftain Savoy cabbage and broccoli. The second experiment employed the same format except that the experimental diets were fed starting 18 days after the last dose of DMH. The results were as follows (ratio of % of mice showing neoplasia of the large bowel/controls): Chieftain Savoy cabbage 0.58, Red Acre cabbage 0.84, and Brussels sprouts 2.1. Under conditions of this experiment, Chieftain Savoy cabbage again exerted an inhibitory effect. Red Acre cabbage showed marginal inhibitory capacities and Brussels sprouts increased the incidence of large bowel neoplasia. The data obtained for Chieftain Savoy cabbage in the two experiments above, as well as those obtained previously, indicate that this vegetable can inhibit carcinogen-induced neoplasia under a variety of conditions.

2. Studies of the effects of coffee and tea on glutathione S-transferase activity. Glutathione S-transferase is a major enzyme detoxifying a large number of chemical carcinogens including polycyclic aromatic hydrocarbons. The enzyme system is inducible. Studies have been carrried out on the capacity of powdered green coffee beans and powered black tea leaves added to the diet to induce increased glutathione S-transferase activity. Coffee is a potent inducer enhancing the activity of this system up to five-fold in the mouse liver and eleven-fold in the small intestine. Tea also had an inducing effect, i.e., approximately 2.5-fold in liver and 2-fold in the small bowel, when added at either 10 or 20% in the diet.

3. Effects of coffee on 7,12-dimethylbenz(a)anthracene (DMBA)-induced mammary tumor formation. On the basis of the effects on glutathione S-transferase activity, studies were initiated using DMBA-induced neoplasia of the mammary gland in female Sprague-Dawley rats to determine if green coffee beans inhibit carcinogen-induced neoplasia. Female rats six weeks of age were placed on the experimental diets containing 10% or 20% green coffee beans for two weeks prior to DMBA administration. Results are as follows (ratio of % of rats fed test diets showing mammary neoplasms/controls): coffee beans 10%, 0.70; coffee beans 20%, 0.40. These data indicate that green coffee contains an inhibitor of DMBA-induced mammary neoplasia.

Significance to Biomedical Research and the Program of the Institute: It would be of importance to identify naturally-occurring inhibitors of neoplasia. Such information would be critical for evaluating epidemiological findings of differences in cancer incidence in various population groups. The information also has potential value in prevention of neoplasia in humans.

Proposed Course: The course as outlined in the original contract will be followed: Contract will end September 29, 1981.

Date Contract Initiated: September 30, 1978

Current Annual Level: 0

NEBRASKA, UNIVERSITY OF (NO1-CP-85674)

Title: Prevention of Pancreatic Cancer in Experimental Animals by Retinoids

Contractor's Project Directors: Dr. Parviz Pour
Dr. Diane Birt

Project Officer (NCI): Dr. Carl E. Smith

Objectives: The present study evaluates the ability of several retinoids to inhibit the development of experimental pancreatic cancer. Tumors will be induced in Syrian hamsters with the pancreatic carcinogen N-nitrosobis-(2-oxopropyl)amine (BOP), and retinoids will be fed in the diet beginning one week after carcinogen treatment. BOP will be administered in a single dose at a low (10 mg/kg BW) or a high (40 mg/kg BW) level, each of four retinoids (13 cis retinoic acid, N-ethyl retinamide, 2-OH ethyl retinamide, 4-OH phenyl retinamide) will be fed at several levels. Body weights, food consumption, clinical chemistry and circulating retinoids will be measured. Histopathology will be evaluated in the pancreas, lung, liver and kidney.

Major Findings: Studies with the high dose of BOP (40 mg/kg BW) and low levels of the three retinoids (0.05, 0.10, and 0.20 mM/kg diet) showed an inhibition of pancreatic adenomas and tumors in the gall bladder and liver in female hamsters. Carcinoma yield in both sexes, and adenoma incidence in male hamsters were not modified by retinoid treatment.

Studies utilizing higher levels of retinoid and lower levels of BOP have been conducted and are being prepared for histopathological evaluation.

Significance to Biomedical Research and the Program of the Institute: Inhibition of cancer by retinoids has proven successful in several experimental models, in particular, in the breast and bladder. This contract studies the effect of these compounds on experimental pancreatic cancer. The inhibition and prevention of cancer is the most logical future course for cancer research.

Proposed Course: Histopathologic evaluation will be completed and all studies will be published.

Date Contract Initiated: September 30, 1978

Current Annual Level: $73,426

OREGON STATE UNIVERSITY (NO1-CP-85660)

Title: Studies of Natural Inhibitors of Chemical Carcinogens

Contractor's Project Director: Russell O. Sinnhuber

Project Director (NCI): Dr. Carl E. Smith

Objectives:
I. Evaluate a number of naturally occurring substances which have been reported to have anti-carcinogenic activity.

2. Determine their mode of action. Aflatoxin B_1 (AFB_1), a powerful liver carcinogen, would be used with the rainbow trout as the test animal. The natural food components which will be investigated are members of the Cruciferae family which includes Brussels sprouts, broccoli and cauliflower. Certain natural flavones and related compounds which have been reported to have similar activity will also be examined in this test system. These include flavone, tangaretin, nobilitin, β-napthoflavone and indole-3-carbinol.

Major Findings: Major findings are discussed under the following categories:

1. Examination of the influence of selected dietary inhibitors on the levels of xenobiotic metabolizing enzymes in vitro.

2. Examination of the influence of such changes, if any, on AFB_1 metabolism and DNA binding in vitro and in vivo.

3. Determination of the correlations, if any, between the above effects and alterations in AFB_1-induced hepatocarcinogenesis.

The first category, the enzyme induction studies, has been completed using broccoli, cauliflower, Brussels sprouts, benzoisothiocyanate, phenethyl isothiocyanate, β-napthoflavone, tangaretin, β-ionone, flavone, indole-3-carbinol, and quercetin in the diet. Activities measured in vitro were cytochromes P450, benzo(a)pyrene monooxygenase, ethoxycoumarin deethylase, ethoxyresorufin deethylase, aflatoxicol dehydrogenase, and aflatoxin M_1 formation. The first four components were found to induce, at most, only marginal changes in enzyme levels. In contrast, the other six components produced changes ranging from striking enzyme induction for a few compounds to apparent depression of some enzymes by others.

The second category, which examines changes in AFB_1 metabolism and DNA binding, is partially completed. Assays using tissue extracts have shown that dietary alterations in enzyme levels may indeed be accompanied by changes in the relative rates of AFB_1 metabolism to aflatoxicol or to aflatoxin M_1. However, assays using intact isolated hepatocytes are expected to provide more meaningful and complete information of AFB_1 metabolism and DNA binding. The trout hepatocyte isolation method has now been characterized with regard to reproducibility of preparation, viability of aflatoxin metabolism and DNA adduct formation, and identification of important variables such as dose-response, AFB_1 and solvent toxicity, and cell concentration effects. Comparative studies using hepatocytes from fish on control versus inhibitor diets are now in progress. Inhibitor studies were also initiated using coho salmon, which have been shown to be more resistant than trout to AFB_1 carcinogenesis. Cohos showed lower levels of procarcinogen activating enzymes but similar levels of detoxifying activities, compared to trout. Cohos also showed much lower binding of i.p. injected ^3H-AFB_1 to liver DNA, suggesting possibly a lower level of tumor initiation in coho for a given exposure to AFB_1. Coho were shown to respond to β napthoflavone as a dietary inducer of xenobiotic metabolizing enzymes. For trout, this was shown to result in decreased binding of i.p. injected AFB_1 in vivo. Comparable studies are planned for coho.

The third category, assessing the influence of putative inhibitors of AFB_1 -induced hepatocarcinogenesis in trout, will be completed by July 1981. Results to date show that dietary components which fail to elicit changes in enzyme levels also provide no protection against high levels of aflatoxin carcinogenesis. However, the tumor incidences observed in this experiment were high (approximately 75%), possibly

masking or overwhelming any modest degree of protection which might occur at lower carcinogen exposure. Some of these trials should therefore be repeated at lower AFB_1 levels. For the trials currently underway, it will be especially interesting to see whether components which do alter enzyme levels and/or aflatoxin metabolism also necessarily show protection against or enhancement of aflatoxin carcinogenesis.

In summary, the results so far indicate that:

1. Young trout on control diet have low basal levels of xenobiotic metabolizing enzymes compared to Fischer rats.

2. These enzymes can be variously induced or repressed in trout by putative dietary inhibitors, with a spectrum of responsiveness partially overlapping that of the rat.

3. Where studied, such changes are accompanied by alterations in aflatoxin metabolism in vitro or DNA binding in vivo.

4. Dietary treatments which fail to alter enzyme levels also fail to alter aflatoxin-induced liver cancer in trout.

Significance to Biomedical Research and the Program of the Institute: Aflatoxin B_1, reported to be the most potent liver carcinogen ever described, is present in many of our foods, yet the incidence of human liver cancer is relatively low. It is conceivable that natural substances in our food are partly responsible for the limited occurrence of this form of cancer by stimulating enzymes which detoxify aflatoxins, and perhaps other potential carcinogens. This project investigates this hypothesis.

The aim of these studies is to investigate the relationship between enzyme induction and tumor response in the trout model, to identify the classes of natural compounds which cause these responses, and to examine the mechanisms by which they operate. Compounds (if any) which show consistent protective effects in a variety of animal models and exposure protocols may then receive priority consideration for further study on possible protective effects in man.

Results to date have shown that trout are sensitive to a range of carcinogens, that they possess a spectrum of xenobiotic metabolism enzyme systems similar to other vertebrates including man, and that these enzymes in trout show responses to various putative dietary inhibitors ranging from marked enzyme induction to substantial depression in some cases. Completion of the tumor incidence studies should therefore provide unique and important information as to whether dietary "inhibitors" are generally effective at ameliorating aflatoxin carcinogenesis or whether, through enzyme repression, some of these compounds may actually enhance the carcinogenic response. Completion of the hepatocyte studies should provide unique information on the mechanism(s) by which these inhibitors operate. These studies involve the simultaneous determination of relative rates of metabolism of AFB_1 to all its end products, including DNA adducts, from individuals on various diets. Although it has been shown that dietary inhibitors can induce enzyme changes, it is not possible to translate this information directly into predictions about tumor response; the various reaction rates along the branched xenobiotic pathway must be determined experimentally. Hopefully, the approach under study will permit this.

Finally, the comparative experiments between coho salmon and rainbow trout hold special promise as an informative model for assessing the importance of dietary

inhibition of aflatoxin carcinogenesis. One genus is sensitive to AFB_1, the other resistant, yet both have been shown to respond markedly to the dietary enzyme inducer, β-napthoflavone on AFB_1 response.

Proposed Course: Hepatocytes will be prepared from fish on selected inhibitor diets and their patterns of AFB_1 metabolism, detoxication, and DNA adduct formation will be compared to controls. These studies, combined with the above enzyme induction/repression work, are aimed at providing a clearer picture of the relative importance of the various enzyme pathways of xenobiotic metabolism, of the mechanisms of aflatoxin carcinogenesis, and of the mechanisms by which various dietary "inhibitors" might influence this process. Contract will end September 29, 1981.

Date Contract Initiated: September 30, 1978

Current Annual Level: 0

RESEARCH TRIANGLE INSTITUTE (NO1-CP-75932)

Title: Synthesis of New Retinoids for In Vitro Studies of Prevention of Lung Cancer and Other Epithelial Cancers

Contractor's Project Director: Dr. F. Ivy Carroll

Project Officer (NCI): Dr. Carl E. Smith

Objectives: The objective of this project is to synthesize retinoids possessing:

1. Effectiveness in controlling cellular differentiation of epithelial tissues.

2. Low toxicity.

3. Low tendency for accumulation. To this end, and based on published information on the subject, a program was developed which proposed the synthesis and testing of the following classes of retinoids:

   a. Derivatives of 13-cis-retinoids.

   b. Cyclic derivatives of all trans retinoids.

   c. All trans retinoids substituted in the polyene side chain.

   d. Analogs of retinyl amine.

Major Findings: During the past twelve-month period, four new retinoids were submitted for testing in the tracheal organ culture (TOC) at NCI. These constituted a continuation of the contractor's investigation of the effectiveness of 12-substituted retinoids as cancer chemoprophylactics. The following compounds were synthesized, characterized and submitted to NCI for testing in the TOC screen:

1. 11,13 -dicis-12-Hydroxymethylretinoic Acid, δ-Lactone.

2. 13-cis-12-Carboxyretinol, δ-Lactone.

3. 13-cis-12-Hydroxymethylretinoil, Diethyl Ether.

4. 12-cis-13,14-Benz-13-desmethyl-12-carboxyretinoic Acid Anhydride

The lactones 1 and 2 complete the set of isomeric lactones of 13-cis-and 11,13-dicis-12-hydroxymethylretinoic acid and 13-cis-and 11,13-dicis-12-carboxyretinol. Comparison of the activity of these four lactones should provide information on the effect of geometric and positional isomerism on retinoid activity. Similarly, comparison of the activity of 13-cis-12-carboxyretinoil, δ-lactone (2) with that of 12-hydroxymethylretinoil, diethyl ether (3) and of retinyl ethyl ether, submitted in the first contract year, will elucidate the effect of a carboxy group vs. that of an ether group on the activity of a derivative of 13-cis-retinol. Furthermore, comparison with the activity of 13-cis-12-hydroxymethylretinoi, previously submitted, will provide direct information of the effect of an ethyl ether vs. a hydroxyl group. Finally, incorporation of the double bond at C-13 in an aromatic ring is tested by comparison of the activity of the "benz" analog of the previously submitted anhydride of 13-cis-12-carboxyretinoic acid. If such a modification were to lead to active compounds, a whole series of 13-cis-13,14-benz-13-desmethylretinoids could be prepared and tested.

Significance to Biomedical Research and the Program of the Institute:
In principle, the program is designed to provide structure-activity correlations relating to the effects of 1) geometric isomerism in the polyene side chain, 2) oxidation state, conformation and configuration of the end group in retinoids, and 3) N-substitution of retinylamine. Such a correlation will aid in the understanding of the mechanism of action of retinoids in cancer prevention and regression as well as to the design and synthesis of retinoids with desirable properties.

Proposed Course: Contract ended, January 29, 1981

Date Contract Initiated: September 30, 1977

Current Annual Level: 0

SOUTHERN RESEARCH INSTITUTE (NO1-CP-85615)

Title: Studies on Toxicology of Retinoids

Contractor's Project Director: Dr. E. Paul Denine

Project Officer: Dr. Carl E. Smith

Objective: To evaluate and compare the relative in vivo toxicity of selected natural and synthetic retinoids.

Major Findings: Studies were conducted to compare the relative in vivo toxicity of all-trans- and 13-cis-isomers of retinoic acid, N-ethyl-retinamide, N-(2-hydroxyethyl)retinamide, and N-(4-hydroxyphenyl) retinamide. Eqimolar concentrations of each isomer were administered either intraperitoneally or orally by gavage to rats and mice for 21 consecutive days or for 12 weeks (oral administration only). In each study, the all-trans and 13-cis-retinoids were administered concurrently. The results of these studies indicate clearly that there are substantial differences in toxicity between retinoids, between all-trans- and 13-cis-isomers, and between routes of administration.

In general, it can be concluded that the ip route of administration results in greater intoxication than the oral route and that the all-trans-isomers are more toxic than the 13-cis-isomer. However, N-ethylretinamide proved to be an exception to the latter. The 13-cis-isomer was more toxic than the all-trans-isomer. A dose related anemia was observed with all retinoids. An increase in plasma alkaline phosphatase activity as well as bone breakage was observed. Hisological examination of the tissues taken at the time of necropsy indicated that the tissue most affected was the liver. The lesions observed included vacuolization, homogeneous degeneration and enlargement of portally distributed hepatocytes, and hepatocyte necrosis. Some male animals exhibited testicular lesions characterized by spermatogenic arrest and mild degeneration and necrosis of the semineferous tubular epithelium.

Significance to Biomedical Research and the Program of the Institute: Results of these studies indicate that, in general, the 13-cis-isomer of a retinoid is less toxic than the all-trans-isomer. This would suggest that the 13-cis-isomer of a very biologically active but toxic all-trans-retinoid might be synthesized which would retain its biological potency, but have decreased toxicity. This compound would be likely to have potential for use as a chemopreventive agent against certain forms of cancer.

Proposed Course: Studies are scheduled to continue to evaluate the relative toxicity of various retinoids. In particular, planned studies include an evaluation of all-trans-N-(2-hydroxypropyl)-retinamide, all-trans-N-(3-hydroxypropyl) retinamide, all-trans-N-(2-hydroxyethyl) retinamide, and the all-trans- and 13-cis-isomers of N-butylretinamide and N-(4-hydroxybutyl) retinamide.

Date Contract Initiated: September 30, 1978

Current Annual Level: $257,781

SRI INTERNATIONAL (NO1-CP-05600)

Title: Synthesis of New Retinoids for the Chemoprevention of Epithelial Cancer

Contractor's Project Director: Dr. Marcia I. Dawson

Project Officer (NCI): Dr. Carl E. Smith

Objectives: To synthesize retinoids that may have better pharmacological properties than those that have already been tested. These analogs have both steric and electronic modifications in the side chain and polar terminus of the retinoid skeleton.

Major Findings: During the past seven months the following retinoid analogs have been prepared:

1. Compounds in which the polar terminus of retinoic acid has been modified by acylation with pentaerythritol and monobenzalpenterythritol.

2. Compounds in which the (11E,13E)-15-CO^2H moiety of retinoic acid has been replaced by 5-carboethoxythiophen-2-yl and 5-carboxythiophen-2-yl groups.

3. Compound in which the (9E,11E,12E)-9-CH^3-15-CO^2H moiety of retinoic acid has been replaced by a 2-formylnaphth-6-yl group.

The biological testing of some of these compounds is in progress. The compound name, given first, is followed by the result of the hamster-tracheal-organ culture assay for reversal of keratinization, which is expressed as the percentage of cultures lacking keratin and keratohyaline granules of retinoid concentrations of 10^{-10} M and 10^{-9} M (a). This assay was conducted by Dr. L. J. Schiff at IIT Research Institute, Chicago, Illinois. Next is listed the effect of the retinoid on the inhibition of the induction of ornithine decarboxylase by 12-O-tetradecanoyphorbol 13-acetate in mouse skin, which is expressed as the percentage of enzyme inhibition at two dose levels, 1.7 and 17 nmoles (b). This assay was conducted at SRI. The preliminary results are presented below:

Pentaerythritol monoretinoate: b) 24%, 80%.

2^{α} Phenyl-5^{β}-hydroxymethyl-5^{α}-retinoyloxymethyl-1,3-dioxane:
b) 26%, 47%.

2^{α}-Phenyl-5^{α}-hydroxymethyl-5^{β}-retinoyloxymethyl-1,3-dioxane:
b) 0%, 37%.

(1Z,3E)-1-(5-Carboethoxythiophen-2-yl)-2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-1,3-butadiene: a) 36%, 43%; b) 35%, 76%.

(1E,3E)-1-(5-Carboethoxythiophen-2-yl)-2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-1,3-butadiene: a) 43%, 71%; b) 67%, 77%.

(E)-Retinoic Acid: a) 65%, 93%; b) 87-91% (1.7 nmoles).

The following compounds have also been submitted for testing:

(1E,3E)-1-(5-Carboxythiophen-2-yl)-2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-1,3-butadiene.

(E)-6-(2-(2,6,6-Trimethyl-1-cyclohexen-1-yl)ethen-1-yl)-2-naphthaldehyde.

Significance to Biomedical Research and the Program of the Institute: Retinoid deficiency enhances the susceptibility of the epithelial tissue of the colon, bladder, and lung of experimental animals to chemical carcinogenesis. Synthetic retinoids can inhibit the development of epithelial cancer of the skin, respiratory tract, mammary gland, and bladder in experimental animals and can reverse the hyperplasia induced by chemical carcinogens in prostatic and tracheal organ cultures. Drug development in this area is required because the prophylactic usefulness of the natural retinoids is limited by their toxicity, tissue distribution pattern, and metabolic deactivation. Synthetic efforts must be aimed at developing nontoxic drugs that could be administered regularly to augment normally operative body mechanisms that arrest or reverse preneoplastic processes during the progression to invasive maligancy.

Proposed Course: To continue the preparation of retinoid analogs, using all available structure-activity data for the design of future compounds.

Date Contract Initiated: August 30, 1980

Current Annual Level: $95,428

WISCONSIN, UNIVERSITY OF (NO1-CP-75905)

Title: Long-term Studies of Prevention of Epithelial Cancer by Retinoids

Contractor's Project Director: Dr. William A. Croft

Project Officer (NCI)): Dr. Carl E. Smith

Objectives: To determine the effect of retinoids on FANFT-induced urinary bladder cancer.

Major Findings: In one study, groups of 100 female rats (50-60 gm) were fed FANFT at 0.2% in the diet for 10 weeks. One week following termination of FANFT feeding (total dose 1.54 gm), 13-cis-retinoic acid (CRA) as a gelatinized beadlet preparation from Hoffmann-LaRoche (Nutley, NJ) was blended into the diets at 120 or 240 mg/kg and fed until studies end at 50 weeks. No bladder tumors were observed in untreated control rats or rats maintained on the two levels of dietary 13-CRA alone. Of the rats pretreated with FANFT, bladder carcinoma developed in 36% of rats maintained on diet without 13-CRA, in 42% of rats maintained on diet containing 120 mg/kg 13-CRA, and in 47% of rats maintained on diet containing 240 mg/kg 13-CRA. Under these conditions, 13-CRA demonstrated no quantitative inhibitory effects on FANFT carcinogenesis in terms of incidence or grade and stage of lesions, but rather increased incidence in an apparent dose-related manner ($p=0.024$).

In a parallel study, groups of 100 female rats were fed FANFT (total dose 1.54 gm). N-ethyl-retinamide (NER) (327 or 654 mg/kg) or N-(2-hydroxyethyl)retinamide (NHER) (343 or 686 mg/kg) were dissolved in an ethanol:trioctonin vehicle (1:3), blended into the diets, and fed until studies end at 50 weeks. No bladder tumors were observed in untreated control rats or rats maintained on the two levels of dietary NER or NHER alone. Of the rats pretreated with FANFT, bladder carcinoma developed in 53% of rats maintained on diet without NER or NHER, in 63% of rats maintained on diet containing 327 mg/kg NER, and in 78% of rats maintained on diet containing 654

mg/kg NER. Under these conditions, NER demonstrated no quantitative inhibitory effects on FANFT carcinogenesis in terms of incidence or grade and stage of lesions, but rather increased incidence in an apparent dose-related manner (P=0.00002). Similarly, bladder carcinoma developed in 67% of rats maintained on diet containing 343 mg/kg NHER and in 49% of rats maintained on diet containing 686 mg/kg NHER.

Significance to Biomedical Research and the Program of the Institute:
The lack of an inhibitory effect with 13-CRA, NER and NHER is consistent with the results of studies employing retinyl palmitate or retinyl acetate in the FANFT model system and is in contrast to the reported results of studies employing natural and synthetic retinoids in nitrosamine or nitrosamide model systems, indicating that the inhibitory activity of retinoids is carcinogen or carcinogen-class specific. These observations, coupled with the observation that the nature of the observed response to retinoid therapy may also be host and tissue specific, lend an element of complexity to approaches in "chemoprevention", insofar as they pose a formidable number of obstacles to the determination of whether or not retinoid therapy might be relevant and applicable to the individual patient suspected of harboring a preclinical lesion. The question that emerges is whether or not such approaches to the chemotherapy of pre-neoplastic lesions will be any less difficult and any more effective than contemporary approaches to the chemotherapy of frank carcinoma.

At the same time, these observations suggest that FANFT-induced bladder carcinogenesis may proceed through a differing pathway of neoplastic progress, one involving differentiations not as much effected by retinoids as is the pathway involved in nitrosamine carcinogenesis, albeit leading to a similar behavioral endpoint. In other words, these considerations suggest that there is more than one pathway for the neodifferentiation of basal urothelial cells to frank carcinoma and that these pathways may be carcinogen-related.

Proposed Course: To study the influence of vitamin A deficiency on the suspectibility of and neodifferentiation in urinary bladder cancer.

Date Contract Initiated: September 30, 1977

Current Annual Level: 0

SUMMARY REPORT
MOLECULAR CARCINOGENESIS

Research supported under the Molecular Carcinogenesis category focuses on changes in biological macromolecules, cellular structure, ultrastructure, and functions as a result of the effects of carcinogen interaction, the identification of biochemical and molecular markers and properties of transformed cells, the development of carcinogenicity/mutagenicity testing procedures, the mechanism of cell transformation, mutagenesis and genetic damage, the role of DNA repair in carcinogenesis, and enzymes associated with the carcinogenic process. Contracts currently in this category fall into five general areas: (1) the role of DNA repair in carcinogenesis; (2) the development of carcinogenicity testing procedures; (3) markers and properties of transformed cells; (4) carcinogen metabolism in human tissues; and (5) changes in cell membrane structure and function. The major accomplishments of each of these areas are highlighted below:

Role of DNA Repair in Carcinogenesis

Important studies on the role of DNA repair in mutagenesis and carcinogenesis are being conducted by several contractors. It is well established that individuals with certain recessively inherited genetic disorders such as xeroderma pigmentosum (XP), ataxia telangiectasia (AT), Fanconi's anemia (FA), and Bloom's syndrome (BS) have a significantly higher predisposition to cancer. These autosomal recessive disorders are associated with defects in the cell's ability to repair DNA damage. Three contractors are developing methods for the detection of heterozygous carriers of the above genetic disorders. These individuals may also be more cancer prone. The contractors at the Sloan-Kettering Institute for Cancer Research (NO1-CP-85665) have continued to investigate the differential induction of chromosome breaks and sister chromatid exchanges by diepoxybutane as a basis for diagnosing FA and AT homozygotes and heterozygotes. Fibroblasts and lymphoblastoid cell lines have been developed from FA and AT homozygotes and heterozygotes for use in these tests. The reliability of this method for pre- and postnatal diagnosis and carrier detection of FA was confirmed by the studies conducted. Preliminary positive results have been obtained for the detection of AT heterozygotes. At the Johns Hopkins University (NO1-CP-85670) six different DNA repair enzymes have been, or are being, isolated and purified from human placentas in quantities sufficient for use in antibody production. More sensitive radioimmunoassays using these antibodies are being developed in order to study the levels and distribution of these enzymes. The basis of a test for identifying heterozygous carriers of the XP disorder originally proposed by the contractors at the University of Chicago (NO1-CP-85669) was to use a previously reported heterogeneity in the ability of lymphoblastoid cell lines to remove O^6-alkylguanine lesions from their DNA. Two classes of cells were distinguished, one which rapidly removes O^6-methylguanine adducts and one which does not in the short test period used. Since both classes were found among XP cell lines of complementation groups A, C, and D, the XP defect and ability to remove O^6-alkylguanine lesions are apparently independent characteristics. Attention has now shifted to an observed differential sensitivity of cells to growth inhibition by N-acetoxy-N-2-acetylaminofluorene as a means of identifying XP heterozygotes and homozygotes.

The O^6-alkylguanine adduct which results from treatment of cells with alkylating agents is an important lesion since it seems to lead to increased mutagenesis in prokaryotes and has been implicated as a potential carcinogenic lesion. Two contractors are attempting to elucidate the mechanisms of removal of this lesion from DNA. The contractors at DOE/Oak Ridge National Laboratory (YO1-CP-00200) have

synthesized a single-stranded DNA polymer containing ^{3}H-labeled O^{6}-methytlguanine as the only modified and labeled base. This substrate was used to demonstrate the presence of an inducible O^{6}-methylguanine repair activity in E. coli and the presence of a similar activity in rat liver nuclei. Experiments conducted by contractors at the International Agency for Research on Cancer (NO1-CP-55630) using chronic versus single doses of N-nitrosodimethylamine have demonstrated that the repair of O^{6}-methylguanine lesions in rat liver DNA is an inducible activity. This inducible repair activity appears to have a limited and finite capacity to cope with the removal of an increased amount of DNA damage.

Other studies on the characteristics and expression of DNA repair in man are being conducted by two contractors at DOE/Oak Ridge National Laboratory. One investigator (YO1-CP-90203) is attempting to quantitate and characterize the type of DNA damage induced by varying wavelengths of ultraviolet (UV) light. The number and kinds of pyrimidine dimers induced by UV were shown to be related to the DNA base ratio, wavelength of UV light and total dose. Studies on the Rauscher leukemia virus inhibition of postreplication repair in mouse NIH-3T3 cells revealed only small differences from that of normal cells. This is in contrast to results obtained by others using rat kidney cells. A second investigator (YO1-CP-90208) is attempting to genetically dissect the DNA repair systems in man and to identify and map the number and kinds of genes required for DNA repair. Sixty-five human x mouse hybrid clones have been analyzed for the ability to repair DNA damage following UV irradiation. The gene for the ability to repair UV-induced damage appears to reside on human chromosome 3 although some discordant results were obtained. Possible reasons for the discrepancy in the data are being investigated further. Mouse x human hybrids have also been isolated using XP cells from the five complementation groups A through E. The results demonstrated that the defective repair capacity of complementation groups A through D, but not group E, can be complemented by mouse cells. It is suggested that these hybrid cells will provide a valuable probe for the genetic and biochemical analyses of the defective DNA repair in XP cells.

Development of Carcinogenicity Testing Procedures

Several contractors are conducting research aimed at the development of animal, cell culture, and enzymatic test systems for use in detecting potential carcinogens and/or tumor promoters. A hairless mouse model system is being developed for UV radiation and chemical carcinogenesis studies at DOE/Oak Ridge National Laboratory (YO1-CP-90201). Four different autosomal mutations leading to a hairless phenotype have been selected and backcrossed into two inbred mouse strains - BALB/c and C57BL/6. These are now in the fifth backcross generation. The different mutations result in differences in the presence of sebaceous glands, the thickness of skin, and the amount of residual follicular tissue. The mutations, given the provisional names poor fur, fur loss, and bald, were determined to be allelic and not at the hairless locus. From studies using the heterozygous and homozygous near nude mouse mutants, it was concluded that hair follicles are important in skin carcinogenesis.

The effect of model carcinogens on epithelial cell culture systems and nuclear RNA polymerase activities from rat liver are being studied by three contractors. The progressive expression of various early markers of cell transformation, the induction of unscheduled DNA synthesis and the inhibition of enzymatic activity are the end points being examined. The contractors at the University of North Carolina (NO1-CP-55707) have demonstrated that the transformation process in cultured liver epithelial cells consists of a slow, step-wise sequence of cell alterations, beginning with damage and presumably incomplete DNA repair and ending with

tumorigenic cells after a period of a year or more. The sequence of steps in the transformation process were shown to include the following: resistance to the cytotoxic effect of the carcinogen, increased growth rate, increased colony forming efficiency, altered colony morphology, cell membrane modifications, karyotypic and chromosomal changes, anchorage-independent growth, and finally, tumorigenicity upon back transplantation into animals. The intelligent utilization of these sequential stages, once their relationship to tumorigenicity is firmly established, may allow early steps in the sequence to be used as end points in a carcinogenicity test system. Improvements in a carcinogenicity test system using cultured primary adult rat hepatocytes and the measurement of unscheduled DNA synthesis or DNA repair synthesis as an end point have been made by investigators at the University of Wisconsin (NO1-CP-85609). The isolation of cell nuclei following the incorporation of labeled thymidine to measure unscheduled DNA synthesis eliminated a previous problem of high background radioactivity. In addition, DNA repair induced by the carcinogens 2-acetylaminofluorene and methyl methanesulfonate could be enhanced by the addition of nicotinamide, isonicotinamide, and other compounds which inhibit the activity of a tightly bound nuclear enzyme, poly (ADP ribose) polymerase. These two modifications contribute to an increased sensitivity of this carcinogen test system. At the University of Illinois (NO1-CP-95631) the molecular mechanism for the inhibition of nucleolar (ribosomal) and nucleoplasmic RNA synthesis in rat liver of animals treated with selected carcinogens was explored. Aflatoxin B_1, which selectively inhibits RNA polymerase II activity, was shown to also inhibit nucleolar RNA synthesis by affecting RNA chain elongation through its interaction with either the ribosomal DNA template or the regulatory chromosomal proteins. The goal of these studies is to determine whether the inhibition of RNA synthesis can be a useful parameter in a test system for carcinogens.

The development of cancer has been shown to be a multistage process beginning with initiation followed by promotion. Few cell culture systems exist which allow the analysis of initiation and promotion events in vitro. Two contractors have been developing cell culture systems for this purpose, one using fibroblast and one using epithelial cells. At the University of Southern California (NO1-CP-65831) the effects of tumor promoters, nonpromoters, and inhibitors of tumor promotion on 5-azacytidine-induced muscle cell differentiation of C3H/10T1/2 cells were investigated. A good correlation was found between the tumor promoting activity of the compounds and their inhibitory action on cell differentiation, except in the case of phenobarbital. From this and other studies, it was concluded that it is unjustified to use the effects on differentiation in model systems as a means to screen for tumor promoters or to define mechanisms of tumor promotion. To parallel the established mouse skin two-stage tumorigenesis model, the investigators at DOE/Oak Ridge National Laboratory (YO1-CP-70227) have continued studies on the development of a two-stage transformation system using epidermal cells from skin tumor sensitive mice. Following carcinogen treatment and further subculturing for ten passages, the isolated cells gave rise to keratinizing squamous cell carcinomas in nude mice. The BALB/c 3T3 clone A31 feeder layer, on which the epidermal cells grow, was found to be required for the optimal production of secondary keratinizing epidermal colonies.

Markers and Properties of Transformed Cells

It is a generally accepted view that the development of cancer is a multistep process in which new cell populations arise representing stages in the cellular evolution from normal, through initiated, preneoplastic and premalignant to frank neoplasia. The acquisition of various biochemical, molecular, and functional markers as a function of time after carcinogen exposure is being investigated. This

will serve as a means of identifying cells at a particular stage in carcinogenesis. Two contractors at DOE/Oak Ridge National Laboratory are developing in vivo and in vitro techniques for studying the development of neoplasia in respiratory tract epithelium. One contractor (YO1-CP-90207) using a tracheal implant-organ culture-cell culture model has determined that the first alteration in growth behavior of epithelial cells derived from specific lesions in tracheal implants exposed to 7,12-dimethylbenz(a)anthracene (DMBA) is a 10-fold higher growth rate. The second alteration is an ability to survive and be subcultured in a selection medium. Other properties such as anchorage independent growth, induction of multinucleation by cytochalasin B, calcium and growth factor requirements and tumorigenicity are being examined in the nontumorigenic and tumorigenic tracheal epithelial cell lines being developed. The second contractor (YO1-CP-90211) has obtained a dose-response relationship for the induction of tracheal carcinomas by DMBA. The development of the epithelial focus (EF) assay allows the quantitation of emerging carcinogen altered and neoplastic cells following exposure to carcinogens. A marked time and dose effect of carcinogen exposure on the frequency of agarose-positive EF was noted. This in vivo-in vitro system is being used to examine the effects of tumor promoters and X-rays on carcinogen altered cells.

Basic mechanisms of neoplastic transformation of cells are being investigated at the Johns Hopkins University (NO1-CP-55713). Using a clonal subdiploid tumor cell line derived from Syrian hamster embryo (SHE) cells in which an extreme karyotypic variability is a characteristic and tumorigenic X normal diploid SHE cell hybrids, the studies conducted have implicated chromosome variability and random allelic assortment of chromosomes as a potential pathway in neoplastic progression. The generation of aneuploid human cells by exposure to grisofulvin, an agent which perturbs chromosomal segregation without inducing DNA/chromosome breakage, will allow the further investigation of the relationship of this characteristic to neoplastic transformation, recessive gene expression and cell senescence.

Carcinogen Metabolism in Human Tissues

There are currently relatively few studies which allow us to understand potential similarities and differences in the response of experimental animals and humans to chemical carcinogen exposure. Data on the comparative metabolism of carcinogens suggest that it is qualitatively similar in general. More information on the carcinogen metabolizing enzymes of human origin is needed to better understand the role of these enzymes in chemical carcinogenesis. Two contractors are engaged in the isolation and purification of the human polyaromatic hydrocarbon (PAH) metabolizing enzymes and the production of antisera to these enzymes. At the University of Texas (NO1-CP-85671) three enzymes involved in the detoxification pathway of PAH metabolism have been isolated from human liver. Two forms of glutathione S-transferase and a single form of phenolsulfotransferase have been purified to apparent homogeneity. Antisera from rabbits is being prepared using these enzymes. The third enzyme, UDP-glucuronyl transferase, has been purified to about 90% purity. Investigators at Vanderbilt University (NO1-CP-85672) have concentrated their studies on human liver cytochrome P-450, NADPH-cytochrome P-450 reductase, and epoxide hydrolase. All enzymes have been purified to electrophoretic homogeneity. Antisera has been prepared which is specific for cytochrome P-450 and epoxide hydrolase. These have been used in comparative interspecies studies. Also, a sensitive immuno-electrophoretic method is being developed for the separation and identification of the human liver enzymes in crude protein mixtures.

At the International Agency for Research on Cancer (NO1-CP-55630) interindividual differences in oxidative benzo(a)pyrene metabolism was studied using normal and

tumorous lung tissue specimens from 105 lung cancer patients. A more than 20-fold interindividual variation in aryl hydrocarbon hydroxylase (AHH) activity was found in both the normal and tumorous lung tissue samples investigated. Consistently lower AHH activity was observed in malignant lung tissue.

Changes in Cell Membrane Structure and Function

Alterations of cell membrane structure, properties and function are known to occur in cells exposed to phorbol ester tumor promoters or transformed by chemical or viral agents. The ability of phorbol ester tumor promoters to induce or inhibit cell differentiation, depending on the cell types being examined, appears to be mediated by the nature of its binding to cell surface membranes. Two contractors are investigating various physiological changes in cells which accompany tumor promoter induced or inhibited differentiation. Investigators at DOE/Oak Ridge National Laboratory (Y01-CP-70222) are examining tumor promoter induced terminal differentiation in human HL-60 promyelocytic leukemia cells. The previously demonstrated changes in cell morphology and lipid biosynthesis which occur during phorbol ester induced differentiation suggested that the primary site of phorbol ester action was the cell surface. Analysis of the specific binding of [^{3}H]-phorbol-12,13-dibutyrate (PDBu) in HL-60 cells and in R-35 variant cells, which were selected for their resistance to the action of phorbol esters, demonstrated that phorbol ester induced cell differentiation is due to the down regulation or loss of bound PDBu following maximal specific binding.

Another contractor at DOE/Oak Ridge National Laboratory (Y01-CP-90205) is examining changes in sugar transport and distribution of intracellular calcium following the interaction of cells with the tumor promoter 12-O-tetradecanoyl-phorbol-13-acetate (TPA). The pig kidney epithelial cells used in this study grow and differentiate in culture with the ability to concentrate sugars from the medium. The rate of differentiation is accelerated by compounds that elevate intracellular cyclic AMP, but is inhibited by TPA. Treatment of cells with TPA leads to the redistribution of intracellular calcium from a sequestered to a more free state.

MOLECULAR CARCINOGENESIS

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CHICAGO, UNIVERSITY OF (NO1-CP-85669)

Title: Identification of Heterozygous Carriers of DNA Repair Defects

Contractor's Project Director: Dr. Bernard S. Strauss

Project Officer (NCI): Dr. David G. Longfellow

Objectives: To develop a test for the identification of heterozygotes of human DNA repair deficiency syndromes in order to permit the unequivocal recognition of such individuals. The differential ability of cultured lymphoblastoid cell lines to remove O^6-methylguanine lesions from their DNA was to form the basis of this test.

Major Findings: The removal of O^6-methylguanine (O^6MeG) from the DNA of human lymphoblastoid cells is governed by a system somewhat different from other DNA repair processes. Investigation of this repair process requires high specific radioactivity in the N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) used to produce O^6MeG, since the removal system is saturated at micromolar concentrations. In contrast to the removal of 3-methyladenine induced by alkylation, the O^6MeG removal system behaves as though it is "used up" by low concentrations of adduct. Once exhausted, relatively long periods (of the order of one cell division) are required for regeneration. No evidence for an inducible process has as yet been found.

Lymphoblastoid cells differ in their ability to remove the O^6MeG adduct. Two major classes of cells were distinguished: mex^+ which are able to remove the O^6MeG adduct with a half life somewhere between 10 and 30 minutes, and mex^-, unable to remove detectable amounts of O^6MeG in short periods of incubation. Although it had been reported that xeroderma pigmentosum lines were deficient in their ability to remove O^6MeG, both mex^+ and mex^- lines can be found among xeroderma complementation groups A, C and D indicating that the two characteristics are independent. It is not yet clear whether the difference between mex^+ and mex^- lines is genetic, due to a major polymorphism or developmental, due perhaps to a "turn off" in methyl removing ability in the mex^- strains. The previous reports from other laboratories indicating that liver is proficient in O^6MeG removal whereas brain is deficient, provide a reason for supposing that the difference is epigenetic.

It has been observed that xeroderma heterozygotes are more sensitive to growth inhibition by N-acetoxy-N-2-acetylaminofluorene (AAAF) than their normal counterparts. This differential sensitivity does not extend to differences in sensitivity to UV in one case at least: a heterozygote that was more sensitive to growth inhibition by AAAF showed the same response as its normal counterpart to UV irradiation. The contractor has attempted to use this differential growth sensitivity to AAAF to recognize xeroderma pigmentosum heterozygotes and homozygotes. The test now being evaluated involves a determination of DNA excision repair to identify xeroderma homozygotes and a measurement of growth rate after treatment with AAAF to separate "normal" from xeroderma heterozygotes. The contractor is now attempting to evaluate the methodology by a "blind" study of a series of human lymphoblastoid lines conducted with the cooperation of Dr. Kenneth Kraemer of the National Cancer Institute.

Significance to Biomedical Research and the Program of the Institute:
The hypothesis on which a major portion of carcinogenesis research is based, that the initiating event in tumor formation is the result of combination of some environmentally significant substance with DNA, is susceptible to epidemiological test and the tests would be greatly increased in sensitivity if good methods for the detection of heterozygotes were available. The discovery of heterogeneity in the ability of cell lines to remove the O^6-methylguanine adduct produced by methylating agents provides an additional opportunity. There is much evidence that the O^6MeG adduct plays a major role in carcinogenesis and the differential sensitivity of organs to carcinogenic stimuli has been related to their ability to remove this adduct. One wonders what the developmental mechanism is that turns off the removal system. Understanding of this signal could bring a method of turning the system "on," thereby reducing the susceptibility of particular organs to carcinogens. The availability of cell lines which mimic the difference between different organs provides a unique experimental tool for the investigation of this phenomenon.

Proposed Course: The contractor plans to continue the "blind" study for the identification of xeroderma heterozygotes in an effort to increase the speed with which the growth tests can be done and to understand the biological basis for the test. The contractor plans to determine the relative growth effects of both UV and of AAAF on a number of lines to determine whether there is a differential effect of chemical carcinogens on xeroderma lines.

Date Contract Initiated: September 30, 1978

Current Annual Level: $129,977

DEPARTMENT OF ENERGY/OAK RIDGE NATIONAL LABORATORY (YO1-CP-00200)

Title: A Novel Approach to the Investigation of the Role of DNA Repair in Chemical Carcinogenesis

Contractor's Project Director: Dr. Sankar Mitra

Project Officer (NCI): Dr. David G. Longfellow

Objectives:
1. To elucidate the mechanism(s) of removal of O^6-alkylguanine from DNA in rat liver.

2. To purify and characterize the repair enzyme(s) involved.

3. To investigate the inducibility of the repair enzyme(s) in rat liver.

Major Findings: A single-stranded DNA polymer containing O^6-methyl[8-^{3}H]guanine (10 mCi/mmol) was synthesized by a series of chemical and enzymatic steps and used as a substrate for investigations of O^6-MeG repair in a cell-free system. Initial experiments were conducted with extract of E. coli in which a high level of O^6-MeG repair activity can be induced by exposure of the cells to N-methyl-N'-nitro-N-nitrosoguanidine. This inducible activity was shown to cause in situ demethylation of O^6-MeG resulting in the formation of [^{3}H]dG in the synthetic DNA substrate.

An extract of rat liver nuclei was shown to cause the removal of [CH_3-^{3}H]O^6-MeG from DNA which had been alkylated with N-[^{3}H]methyl-N-nitrosourea (1.6 Ci/mmol). The level of activity was approximately 1,000 times less than that in induced E. coli extract, and an O^6-methyl[8-^{3}H]guanine-containing DNA substrate of high specific activity will be required in order to determine whether this repair process also involves in situ demethylation.

Significance to Biomedical Research and the Program of the Institute: The demonstration of in situ demethylation of O^6-MeG by extracts of induced E. coli provides the first unequivocal evidence for this repair pathway in a living organism, although it is not yet certain that the same pathway is operative in mammalian cells. Because of the proposed mutagenic and carcinogenic role of O^6-alkylguanine lesions in DNA, studies of the mechanism, efficiency, and inducibility of its repair are important to the understanding of the carcinogenic action of simple alkylating agents.

Proposed Course: A DNA substrate containing O^6-methyl[8-^{3}H]guanine at high specific activity (> 1 Ci/mmol) will be synthesized and used as a substrate to determine whether in situ demethylation occurs in the presence of rat liver extracts, or whether the removal of O^6-MeG involves a different repair pathway, such as base or nucleotide excision. After the repair pathway has been determined, appropriate assay methods will be used to purify the repair enzyme(s), which will then be characterized with respect to general properties, substrate specificity, and mode of action. The inducibility of the enzyme(s) in rat liver by treatment with alkylating carcinogens will also be investigated.

Date Contract Initiated: July 1, 1980

Current Annual Level: $36,500

DEPARTMENT OF ENERGY/OAK RIDGE NATIONAL LABORATORY (YO1-CP-70222)

Title: Malignant Cell Transformation and Mutagenesis Induced by Carcinogenic Chemicals

Contractor's Project Director: Dr. Eliezer Huberman

Project Officer (NCI): Dr. Paul Okano

Objectives:
1. To isolate mutagen-resistant cells for mutagenesis and DNA repair studies.

2. To study the induction of different markers of differentiation in human malignant cells by tumor promoting agents.

3. To study the alterations in lipid biosynthesis in differentiating human tumor cells.

Major Findings: For specific DNA repair processes to be linked with mutagenesis and cell recovery in mammalian cells, there is a need for a variety of DNA repair mutants in addition to the few now known, e.g., xeroderma pigmentosum cells. Of interest is a cell variant designated as VR-43 which was derived from the Chinese hamster V79 cells. These VR-43 cells express, after treatment with methylating but

not with ethylating mutagens, an enhanced postreplication repair compared to the parent V79 cells. Furthermore, after treatment with methylating but not with ethylating agents or X-rays, they exhibit an increased cell survival and at equicytotoxic doses, higher mutation frequencies for both ouabain or 6-thioguanine resistance. Based on these experiments, it can be suggested that after treatment of mammalian cells with some methylating agents, postreplication repair can cope with DNA lesions responsible for cytotoxicity, and to a lesser degree, with lesions responsible for mutagenicity.

Phorbol-12-myristate-13-acetate (PMA) and related phorbol diesters, which are tumor promoters in a two-stage mouse skin carcinogenesis system, can induce cell differentiation in some human cell types, including the HL-60 promyelocytic leukemia cells at doses as low as 10^{-10}M. Cell differentiation can be characterized by an inhibition of cell growth and by increases in the per cent of morphologically mature cells, phagocytosis and lysozyme activity. The HL-60 cells thus represent a useful tool by which some known tumor promoting agents can be studied.

Changes in cell morphology and in lipid biosynthesis, in the HL-60 cells, lend themselves to the suggestion that the primary site of PMA action in the HL-60 cells is the cell surface. It was, therefore, of interest to determine whether indeed phorbol-diester receptors are involved in the biological effect of these agents in the HL-60 cells. To accomplish this, specific [^{3}H]-phorbol-12,13-dibutyrate (PDBu) binding was analyzed in R-35 and HL-60 cells. R-35 variant cells were derived from HL-60 cells previously subcultured 35 times in the presence of 5×10^{-10} PMA. The R-35, unlike the HL-60 cells, did not undergo differentiation following exposure to either PMA or PDBu at doses up to 3×10^{-7} M. The variance in the response of HL-60 and R-35 cells to PMA or PDBu-induced cell differentiation was not due to either the number of binding sites or the affinity of [^{3}H]-PDBu specific binding, but rather to the down regulation (i.e., loss of PDBu bound to the cells) of bound PDBu following maximal specific binding. This was deduced from the fact that the down regulation of bound [^{3}H]-PDBu seen in HL-60 was not observed in R-35 cells.

Based on these and other studies, it can be suggested that down regulation of specific PDBu binding is an important phenomenon involved in the mechanism of action of phorbol esters that leads to the alteration in cellular differentiation of HL-60 cells and perhaps other biochemical and biological events in these and perhaps other cell types.

Significance to Biomedical Research and the Program of the Institute:
Our current state of knowledge suggests that carcinogenesis is a multistage process which begins with an initial mutational event and requires further steps of a nonmutational nature,i.e., promotion. Phorbol diesters such as PMA have been shown to be active tumor promoters in a two-stage (initiation-promotion) mouse skin carcinogenesis model. The exact mechanism by which these chemicals promote tumor formation is unknown. PMA has also been found to alter cell differentiation in some avian, murine, and human cells. The induction or inhibition of terminal differentiation in these cells is related to the tumor promoting activity on mouse skin. Studies of the induction of various membrane and biochemical changes which occur during the induction of terminal differentiation in the human HL-60 promyelocytic leukemia cell system will help in elucidating the cellular controls involved. The mechanisms by which phorbol diesters control cell growth and cell differentiation may be relevant to our understanding of processes involved in tumor promotion by chemicals.

Proposed Course:
1. To study the types of cellular blocks involved in the induction of terminal differentiation in human HL-60 promyelocytic leukemia cells by tumor promoting phorbol diesters.

2. To analyze changes in lipid biosynthesis involved in the process of induction of terminal differentiation by phorbol diesters.

3. To analyze the possible reversible stages or steps in the commitment of HL-60 cells to PMA-induced terminal differentiation.

4. To study the induction of terminal differentiation in HL-60 cells by teleocidin.

Date Contract Initiated: September 30, 1977

Current Annual Level: $256,500

DEPARTMENT OF ENERGY/OAK RIDGE NATIONAL LABORATORY (YO1-CP-70227)

Title: In Vitro Transformation of Tumor Sensitive Epidermal Cells: A Bioassy and a Model for the Study of the Mechanism of Action of Tumor Initiators and Promoters

Contractor's Project Director: Dr. Thomas J. Slaga

Project Officer (NCI): Dr. Paul Okano

Objectives: To develop a reliable and quantitative in vitro transformation system using epidermal cells from skin tumor sensitive mice which will be a relevant one in which two-stage transformation is operational using phorbol ester tumor promoters. Specifically, the contractor plans:

1. To compare transformation of primary or secondary cultures of newborn and adult epidermal cells using much improved culture conditions which allow the epidermal cells to grow and differentiate for a long period in culture much as they do in vivo.

2. To perform similar transformation studies with one of the isolated non-tumorigenic epidermal cell lines which has a faster growth rate.

3. To determine the effects of inhibitors of tumor initiation and promotion in the cell culture systems.

Major Findings: Using primary epidermal cells, several epidermal cell lines were obtained after B(a)P, MNNG and B(a)P-diol-epoxide treatment. These epidermal cell lines have recently been injected into nude mice, but it is too early for the development of tumors. The Seca cell line was also treated with B(a)P, B(a)P-diol-epoxide and MNNG. It was previously reported that when the Seca cell line was exposed at the tenth passage to B(a)P and then subcultured for ten more passages, these cells gave rise to a keratinizing squamous cell carcinoma when injected into a nude mouse. Similar results were also obtained in our repeat experiment using B(a)P, B(a)P-diol-epoxide and MNNG. All the treated cultures after ten passages gave rise to tumors in nude mice. Two out of 6 of the untreated flasks gave rise to

tumorigenic cells after 20 passages. The passaging of the untreated Seca cell line is being continued to see if eventually all flasks contain tumorigenic cells.

The nature of the 3T3 feeder layer effect on epidermal cells in culture was also studied. Mouse epidermal cells can be subcultured at 31° onto an irradiated BALB/c 3T3 clone A31 feeder layer. A31 cells were found to be specifically required for the optimal production of secondary, keratinizing epidermal colonies. This effect was not transmitted through the medium nor by the culture surface, since A31 cells plated on one end of a flask did not stimulate epidermal proliferation at the other end, even if the other end had previously held A31 cells. Epidermal contact with living A31 cells was probably necessary for the effect; killed A31 cells were ineffective. The tumor promoter 12-O-tetradecanoyl-phorbol-13-acetate also blocked epidermal colony formation, probably not through simple toxicity.

It has been shown that SENCAR epidermal cells are more sensitive than BALB/c cells to PAH toxicity in vitro. Cytotoxicity was linear with respect to the logarithm of the carcinogen concentration, a characteristic of many chemical toxicity curves.

In vitro toxicity with several different PAH's [B(a)P, 3-methylcholanthrene, and 7-12-dimethylbenz[a]anthracene] in epidermal cells correlated reasonably well with their established in vivo carcinogenic activities. While not directly involved in carcinogenesis, toxicity may result through a similar mechanism.

Primary epidermal cells, Seca cells, and Seca-transformed cells were also found to contain specific phorbol ester receptors. In addition, these cells were able to metabolize B(a)P to organic solvent soluble and water soluble products in a similar manner.

Significance to Biomedical Research and the Progam of the Institute:
Since the majority (>85%) of human cancers arise from epithelial tissue, it is important to have a quantitative in vitro transformation system using epithelial cells. The question must be asked whether the results obtained from the in vitro transformation of fibroblasts are significant in terms of all forms of cancer and if the results can be extended to transformation studies using epithelial cells. The contractor's studies, therefore, to develop a reliable and quantitative in vitro transformation system using mouse epidermal cells from tumor sensitive mice are highly significant. In addition, it is important that the transformed cells give rise to keratinizing squamous cell carcinomas when injected into a syngeneic host.

Proposed Course: Studies will continue with the aim of establishing an initiation-promotion, two-stage system of in vitro transformation using primary and secondary cultures of epidermal cells from newborn and adult mice and cultures of nontumorigenic epidermal cell lines isolated previously. Known promoters and nonpromoting compounds will be compared for their effect on this system. The effect of known inhibitors of tumor initiation and promotion will be tested as well as specific inhibitors of polyamine and prostaglandin biosynthesis.

Date Contract Initiated: September 30, 1977

Current Annual Level: $185,356

DEPARTMENT OF ENERGY/OAK RIDGE NATIONAL LABORATORY (YO1-CP-90201)

Title: Ultraviolet Radiation Carcinogenesis

Contractor's Project Director: Dr. R. J. Michael Fry

Project Officer (NCI): Dr. David G. Longfellow

Objectives: This project falls into two parts:

1. The development of mouse model systems for the study of ultraviolet radiation (UV) and chemical carcinogenesis and some of the factors that influence carcinogenesis of the skin in particular, and carcinogenesis in general.

2. The investigation of the differences in susceptibility for UV carcinogenesis that are dependent on genotype.

These latter studies are designed to capitalize on the differences between, and the similarities in, two stocks of hairless mice, namely SKH:hairless-1 and HRS/J/Anl.

Major Findings:
1. Four autosomal mutations were selected, all of which show a hairless phenotype but differences in the presence of sebaceous glands, the thickness of the skin and amount of residual follicular tissue. These mutations have been given the following provisional names, poor fur (PF), fur-loss (Frl), near naked (NN) and bald (BL). Good progress has been made in backcrossing the selected mutants to the two inbred strains - BALB/c and C57BL/6. Mice are now being produced from the fifth backcross generations. The fertility and average litter size has remained satisfactory, but in the case of the BALB/c mutants, production is somewhat less in the fifth backcross generation than in the third backcross generation.

The contractor has established that poor fur, fur loss, and bald are allelic, and the mutation is not at the hr locus. Although the three mutants are allelic, there are significant differences in the morphology and cell proliferation of the skin. The major differences between the epidermis of the mutants and the haired BALB/c is the thickness of the epidermis, which approaches the thickness of skin from some parts of the human body. The results for labeling index (fraction of cells in DNA synthesis) indicated that the BL probably has the highest turnover rate of the cells of the basal layer of all the hairless phenotypes including SKH:hairless-1 and HRS/J/Anl mice.

The induction of UV-induced DNA damage was studied using the endonuclease-sensitive sites (ESS) assay. The following results were obtained: BALB/c, 6.8×10^{-11} ESS/dalton/J/m^2, these mice were shaved prior to exposure to UV; SKH:hairless-1 mice, 1×10^{-10} ESS/dalton/J/m^2, 6×10^{-11} ESS/ dalton/J/m^2 in both fur loss and bald mice; and 7.8×10^{-11} ESS/dalton/J/m^2 in the poor fur.

Since one of the future uses of the new mutants with a hairless phenotype is the study of host factors in skin cancer, the immune competence of the mutants was investigated. There has been considerable interest in the role of natural killer (NK) cells in tumorigenesis. It was established that the mutants on C57BL/6 background can be ranked for NK cells (from least to most) in the following order: C57BL/6, Frl, BL, NN, and PF.

2. Another future use of these mutants is the study of the importance of structural differences in the susceptibility for skin cancer induced by UV and chemical carcinogens.

In the case of the NN mice, heterozygous (NN/+) and homozygous (NN/NN) genotypes were produced. The homozygous NN mice are hair deficient from birth, whereas the heterozygous mice develop a partial neonatal pelage that becomes progressively thinner with successive hair growth cycles until only the guard hairs remain. Homozygous and heterozygous NN mice, C3H and C57BL/6 haired mice were tested for susceptibility to B(a)P skin carcinogenesis. The homozygous NN mice were more resistant than mice from any of the other groups, but the partially epilated heterozygous mice were as susceptible as the sensitive inbred C57BL/6. It was concluded that the hair follicle is important in skin carcinogenesis. Since the NN/+ mice, with relatively few follicles, are sensitive as the haired C57BL/6, it appears that only a few follicles are required to increase the sensitivity to chemical carcinogens.

3. The experiments are designed to establish whether HRS/J/Anl and SKH:hairless-1 differ in susceptibility for UV-induced cancer, and if so, whether the differences are due to factors that influence initiation or those that affect expression. The study is still in progress but the results, so far, indicate that the incidence of induced skin tumors is different in the two hairless stocks. When the regimen of UV exposures is followed by treatment with a phorbol ester (TPA), the difference between the two hairless stock is almost eliminated. These results are consistent with those that were previously reported for skin carcinomas induced by 8-MOP plus UVA and suggest strain-dependent differences are largely due to differences in the expression of initiation events.

Significance to Biomedical Research and the Program of the Institute:
Cancer of the skin is by far the commonest tumor in the U.S. white population, and melanoma is one of the few tumors for which the incidence is increasing. It is clear that UV is a major etiological factor and that UV-induced lesions in DNA and their repair are becoming increasingly understood. Thus, UV-induced skin cancer in mice is an appropriate model for investigating the role of specific DNA lesions and their repair in carcinogenesis. As most of the events following exposures to UV that result in cancer are similar in man and mouse, with the exception of excision repair of pyrimidine dimers, these studies will provide data that will elucidate the factors that determine species-dependent differences and possibly suggest methods of extrapolation.

Proposed Course: Included will be the following studies:

1. UV-induced damage and its repair.

2. The cell kinetics of the epidermis.

3. The immune competence of the various mutant stocks in the fifth backcross generation. These assays will also be carried out in the C57BL/6 and BALB/c strains which will establish whether any of the characteristics under study (for example, immune incompetence) are linked with the mutation associated with the hairless phenotype. On the basis of the various investigations, one of the allelic mutants will be selected for further inbreeding. The contractor hopes to freeze embryos of the mutants that will not be used in the immediate future. The UV carcinogenesis experiment that is designed to determine whether strain- and age-dependent

differences in susceptibility to UV-induced skin cancer are due to differences in the initial events or in their expression, a question of general interest in carcinogenesis, will be completed.

Date Contract Initiated: September 30, 1979

Current Annual Level: $179,640

DEPARTMENT OF ENERGY/OAK RIDGE NATIONAL LABORATORY (YO1-CP-90203) (Formerly YO1-CP-50200)

Title: DNA Repair Mechanisms in Carcinogenesis

Contractor's Project Director: Dr. James D. Regan

Project Officer (NCI): Dr. David G. Longfellow

Objectives: The primary objectives are to elucidate the molecular events in human cells when cellular macromolecules such as DNA are damaged by radiation or chemical agents. The Contractor will study and characterize:

1. The sequence of DNA repair events.

2. The various modalities of repair.

3. The genetic inhibition of repair due to mutation.

4. The physiological inhibition of repair due to biochemical inhibitors.

Major Findings: Studies were conducted using a Westinghouse FS40 sunlamp that produces mostly near-ultraviolet light (UV) but some wavelengths in the far-UV as well. The spectral irradiance of the cellulose acetate (Kodacel) filtered lamps closely simulates sunlight. Labeling of both thymidine and cytosine in human cells with [^{14}C]uridine as a precursor, allows quantitation of all three dimers by using two-dimensional paper chromatography. Cytosine-containing dimers make up a larger proportion of the total dimers when cells are irradiated with low biologically relevant doses of the wavelengths contained in sunlight. A low dose of filtered FS40 light produces dimers at a ratio of 20:40:40 (C<>C, C<>T, T<>T) in human cells. The number and kinds of pyrimidine dimers induced in DNA by ultraviolet light are related to the DNA base ratio, the wavelength of UV light and the total dose. In human cells, after low (biological) doses of 254 nm light, the thymine-containing dimers comprise about 75% of the total dimers; after "sunlight" the number is closer to 60%. The results using "sunlight" wavelengths suggest that there is no excision repair sensitivity relative to 254 nm. One may conclude from this that the source of the insult to the cells is not an important factor in determining rate of dimer removal. Moreover, if and when other photoproducts are formed by wavelengths between 290 and 400 nm (e.g., thymine glycols), the possible repair of these photo-products does not inhibit the excision of pyrimidine dimers. The ara-C assay was used to compare repair levels in log-phase cultures of normal and XP-variant human fibroblasts by using succesive three-hour pulses with ara-C and hydroxyurea up to 24 hours after irradiation. The amount of repair, calculated by taking the sum of DNA breaks which accumulated during each pulse, was 70% higher in the XP-variant cell line. Because confluent cultures lose their dependence on

hydroxyurea when the ara-C assay is employed, the pulse assay also was used in such cultures where no additional semi-conservative DNA synthesis was taking place. Although a higher number of single-strand breaks was observed in the normal cell line, the XP-variant showed 25% more breaks.

Viral inhibition of postreplication repair in mouse cells is temperature sensitive even for the wild-type virus. Mouse NIH-3T3 cells were used as hosts and were infected with wild-type Rauscher leukemia virus. These infected cells showed the same level of postreplication repair as did uninfected cells at 31°C. In contrast, these cells showed some inhibition of postreplication repair when rates were measured at 37°C. In addition, other factors appear to affect the level of inhibition observed. The amount of inhibition of postreplication repair in the infected NIH-3T3 cells is small when compared to the level of inhibition originally observed in the normal rat kidney cells by Waters et al. This difference may reflect differences in host cell type, multiplicity of infection or other unknown factors. Unfortunately, the low level of inhibition with the wild-type makes any comparison with the temperature sensitive mutants difficult.

Significance to Biomedical Research and the Program of the Institute:
The significance of these studies lies in:

1. The ubiquitousness of repair (most organisms, including man, have several complex repair systems).

2. The belief that mutagens and carcinogenic events may arise only from residual (nonrepaired) lesions, or that error-prone repair systems may be the major induction mechanisms of the mutagenic or carcinogenic event.

3. The clear association of repair defects and highly carcinogenic disease states in man (xeroderma pigmentosum).

Proposed Course: Further quantitation of all three pyrimidine dimers at other wavelengths will continue. The fact that the XP-variant still has a greater number of repaired regions suggests that it has either a smaller available pool size available for repair synthesis or that there are defective DNA polymerases in the XP-variant which result in greater sensitivity to repair inhibitors. Studies are in process with inhibitors of both α and β DNA polymerases to resolve possible differences between the normal and XP-variant cell.

Measurements of the effect of simian retrovirus infection on the rates of postreplication repair in human cell cultures have been undertaken. Preliminary experiments indicate that simian C-type viruses may also inhibit repair in their host cells. However, a definitive conclusion awaits verification and extension of this observation.

Date Contract Initated: April 1, 1977

Current Annual Level: $129,279

DEPARTMENT OF ENERGY/OAK RIDGE NATIONAL LABORATORY (YO1-CP-90205) (Formerly YO1-CP-50200)

Title: Regulation of Membrane Transport Systems and Membrane Turnover in Carcinogenesis

Contractor's Project Directors: Dr. R. J. M. Fry
Dr. J. S. Cook

Project Officer (NCI): Dr. David G. Longfellow

Objectives: To delineate changes in the function of known and identifiable cell-surface molecules as such changes occur with altered states of cell growth and differentiation, especially in response to treatment of cells with tumor promoters; to delineate the underlying mechanisms responsible for the changes in surface functions.

Major Findings:
1. Regulation of a Surface Transport Protein in HeLa Cells - An essential enzyme of the cell surface is the sodium-potassium transporter that maintains the appropriate levels of these metal ions within the cells; these levels are, in turn, essential to many of the normal functions of all mammalian cells. It has frequently been observed that in neoplastic cells the activity of this transport system is elevated with respect to the normal cell of the same cell lineage. If cells are stressed in various ways so that the normal levels of metal ions cannot be maintained with the existing transport capacity of the surface enzyme, the cells respond with an increase in activity of transport. By several criteria it can be shown that this increase is due to more enzyme molecules present on the cell surface. The enzyme is not a static molecule, but is constantly being removed from the surface (turnover) and replaced by newly synthesized protein; the amount present at any given time is the resultant of the relative rates of synthesis and turnover. In responding to stress, the cells do not change their synthetic rate, but increase their surface activity by decreasing the turnover rate. The molecular basis for this regulation has not been delineated.

2. Recycling of the Surface Membrane - The entire cell surface is constantly turning over. The mechanism appears to involve the pinching-off of surface membrane into small vesicles that are taken into the cell where they are degraded, while other vesicles from within the cell fuse with the surface and supply new membrane. Recent evidence suggests indirectly that some of the membrane that is internalized by the pinching-off process may escape intracellular degradation and be returned to the surface intact. This has now been directly demonstrated by the following experiment. Complex sugar molecules on the cell surface (sialic acids) can be chemically modified to other molecular species that are susceptible to removal by extracellular treatment with specific enzymes (neuraminidase) and are, therefore, identifiable as being on the surface. Several hours after chemical modification, much of the new species is no longer vulnerable to extracellular treatment, and by this and other criteria appears to have been taken into the cell. Hours later, the modified species can again be found on the cell surface. Since the modified species is not synthesized by the cells themselves, and since the modified sugar is on the end of a long chain of molecules that make up the complex surface macromolecule (glycoprotein), the entire complex molecule must be taken into the cell where it is protected from extracellular attack, and later recycled as an intact molecule back to the cell surface.

3. Cell Culture Studies on the Regulation of Sugar Transport in Epithelia - Pig kidney cells (PK_1) grow and differentiate in culture, forming epithelial sheets that have the property of concentrating sugars. In all its characteristics, this property is indistinguishable from the highly differentiated function found in kidney tubules that recovers sugar from the urine of normal animals. In the cultured cells, the development of this differentiated property can be studied over a period of days in cells that can be cloned, i.e., in cell populations that are genetically homogeneous. The rate of differentiation can be accelerated by compounds that either directly (theophylline, methyl isobutylxanthine) or indirectly (hexamethylene bisacetamide) elevate intracellular cyclic adenosine monophosphate (cAMP). Although cAMP appears to be a necessary factor in accelerating differentiation, it can be shown not to be sufficient for terminal differentiation. Significantly, the tumor promotor TPA (tetradecanoyl phorbol acetate) strongly inhibits both the differentiation and the elevation of cAMP. If applied while differentiation is underway, TPA can arrest further normal development and allow the outgrowth of undifferentiated cells.

4. Tumor Promoters and Distribution of Intracellular Calcium - Although cells contain substantial amounts of calcium, most of it is sequestered in various introcellular compartments; the concentration of free, dissolved calcium in the cytoplasm is very low. Many of the known effects of tumor promoters that have been described in the literature could be ascribable to the release of intracellular calcium from sequestered stores with the consequent elevation of the free calcium concentration; this, in turn, could have dramatic consequences for the cells' physiology. By newly developed extensions of known techniques for fractionating cells, it can be shown that treatment of cells with tumor promoters does, in fact, lead to a redistribution of intracellular calcium. The effect is not direct. Tumor promoter (TPA) by unknown means causes the cells to synthesize a new protein(s); the redistribution of calcium is a consequence of this synthesis. The altered physiology of the tumor-promoted cell, including its apparent loss of communication with neighboring cells, is clearly related to its altered calcium metabolism.

Significance to Biomedical Research and the Program of the Institute:
Cells interact with their environment through their surface membranes, and control their own internal environment by the activities of specific transport molecules at their surfaces; in some epithelia (kidney tubules) certain transporters are the manifestation of terminal differentiation of normal cells. Many of these activities are altered in neoplastic cells, including aberrant differentiation in cells treated with tumor promoters. Understanding of mechanisms of these alterations is important to an understanding of the altered physiology of these cells, and of special significance to the understanding of carcinogenic processes is how tumor promoting chemicals modify the normal differentiation of epithelia and allow the continued growth of undifferentiated stem cells.

Proposed Course: Emphasis will continue on the effects of tumor promoters as inhibitors of normal differentiation of kidney epithelial cells; the differentiated function to be studied will continue to be the energy-dependent, concentrative transport of sugars. Recently derived clones of these cells show differential responses to various chemical treatments, and these differences will be exploited in study of mechanisms. The question of whether tumor promoters lead to a breakdown of cell-to-cell communication in epithelia will be tested directly in collaboration with W. Loewenstein (Miami) who has pioneered the appropriate techniques. If the cells can be synchronized, a study of cell-cycle relationships to differentiation and its inhibition by tumor promoters will be pursued.

The preliminary studies on membrane recycling will be made more definitive, and its relationship to control of cell-surface activities by modification of membrane turnover will be further explored.

Date Contract Initiated: April 1, 1979

Current Annual Level: $140,220

DEPARTMENT OF ENERGY/OAK RIDGE NATIONAL LABORATORY (YO1-CP-90207) (Formerly YO1-CP-50200)

Title: Respiratory Carcinogenesis - Markers of Neoplastic Development in the Respiratory System

Contractor's Project Director: Dr. Ann C. Marchok

Project Officer (NCI): Dr. Paul Okano

Objectives:
1. To define specific stages in the evolution of neoplasia in respiratory epithelium.

2. To develop and utilize in vivo-in vitro and in vitro model systems to define and quantitate cellular changes that identify particularly the preneoplastic stages of carcinogenesis.

3. To determine the effects of retinoids on the differentiation and progression of neoplasia in these systems and thereby contribute to the understanding of the mechanism of action of vitamin A.

Major Findings:
1. Using the tracheal implant-organ culture-cell culture model, experiments are in progress to study the growth behavior and progression to neoplasia of epithelial cell populations derived from specific lesions induced in tracheal implants exposed for 2 or 6 months to empty beeswax pellets or beeswax pellets containing 200 µg dimethylbenz(a)anthracene. The specific lesions were identified by cutting the tracheas into 12 equal size explants, placing them in organ culture for 24 hours, and collecting the exfoliated cells in the medium for diagnostic cytopathology. The explants were then placed in outgrowth culture to establish primary epithelial cell populations. The first alteration in growth behavior of the 2 and 6 month carcinogen-preexposed primary cultures was detected as a mean growth-rate 10-fold above control cultures. The second alteration was detected as survival and subculture of 81.3% and 62.5% of the 2 and 6 month preexposed primary cultures, respectively, after being placed in a selection medium in which no control cultures survive. Additional parameters for neoplasia, namely, anchorage-independent growth and tumorigenicity, are currently being tested on the subculturable lines. Also, the growth behavior of the cell populations from the individual explants is now being assessed and matched to the type of lesion on the explants at the time the cultures were initiated to determine if there are direct correlations between these properties and the severity of the lesion.

2. Experiments are continuing with the tracheal organ culture-cell culture model to quantitate the effects of a single dose (2 µg/ml) versus a split dose (4 x 0.5 µg/ml) of benzo(a)pyrene on the initiation of transformation in tracheal organ cultures. At the present time, it has been determined that the split dose increases the incidence and decreases the time to appearance of morphologically altered foci (a preneoplastic marker) in cell populations derived from the carcinogen-exposed tracheal explants. Further tests for the progression of neoplasia (subculturability, growth in 0.33% agarose, multinucleation in the presence of cytochalasin B, tumorigenicity) are currently being tested.

3. The properties of non-tumorigenic and tumorigenic tracheal epithelial cell lines are being studied as part of a program on the development of a rapid, highly quantitative system for studying the control of differentiation and neoplastic transformation in tracheal epithelial cells. Recently, it has been determined that:

a. In contrast to that reported for fibroblast cell lines, both non-tumorigenic and tumorigenic cell lines are highly sensitive to methylglyoxal bis(guanylhydrazone), an inhibitor of S-adenosylmethionine decarboxylase and, therefore, tumorigenic cells cannot be identified by resistance to this inhibitor.

b. Tumorigenic tracheal epithelial cell lines require less calcium in the culture medium for survival.

c. Epidermal growth factor is a potent mitogen in the absence of insulin in the cell lines tested so far.

d. In vitro transformation studies are in progress to study the effects of single versus multidoses of N-methyl-N'-nitro-N-nitrosoguanidine on the frequency of transformation in non-tumorigenic cell lines exposed at different seeding densities.

4. A serum-free defined medium has been developed which supports the growth, differentiation, and continuous subculture of at least two tracheal epithelial cell lines.

Significance to Biomedical Research and the Program of the Institute:
Lung cancer is one of the common diseases in man. Current data, mostly from skin and liver carcinogenesis studies, suggest that the development of cancer is a multistage event. These studies should:

1. Define cellular and biochemical changes in epithelial cells which will mark stages in the progression of neoplasia in respiratory tissue.

2. Identify ways retinoids may alter these cellular processes and thereby alter the progression of neoplasia.

Proposed Course: The experiments in progress will be brought to a conclusion since this contract ends August 31, 1981.

Date Contract Initiated: September 1, 1980

Current Annual Level: 0

DEPARTMENT OF ENERGY/OAK RIDGE NATIONAL LABORATORY (YO1-CP-90208)

Title: Genetic Analysis of DNA Repair in Man with Cell Hybrids

Contractor's Project Director: Dr. Peter A. Lalley

Project Officer (NCI): Dr. David G. Longfellow

Objectives: The long range goal of this project is to investigate the expression and individual genetic components involved in DNA repair in man. Such an elucidation of the genetic basis of DNA repair is fundamental if we are to understand its pivotal role in carcinogenesis. Therefore, the primary objectives of this project will be:

1. To genetically dissect the DNA repair systems in man.

2. To identify the number and kinds of genes required for DNA repair.

3. To assign these genes to specific human chromosomes.

4. To identify and determine the chromosomal assignment(s) of the genetic defect in XP.

Major Findings: Sixty-five human x mouse primary hybrid clones have been analyzed for the ability to repair DNA damage following UV irradiation. These hybrid clones were isolated from five separate fusion experiments employing human cells derived from five unrelated individuals and two different mouse cell lines. Excision repair of UV-induced DNA damage was measured using the BrdU photolysis assay. Using this assay it was shown that mouse cells have 5-10% of the magnitude of excision repair seen in human cells. This result demonstrates that it is possible to distinguish the human DNA repair components from mouse DNA repair components in the human x mouse somatic cell hybrids.

Hybrid cells assayed for excision repair ability appeared to fall into one of three categories:

1. Those having a magnitude of repair similar to human cells.

2. Those having mouse-like repair.

3. Hybrids intermediate between the two.

The segregation of the ability to repair UV damage in the hybrid cells was tested for linkage with 35 enzyme markers representing genes known to be assigned to all the chromosomes except Y. The data indicate that the assignment of a gene or genes required for the ability to repair UV-induced DNA damage can be excluded from all the human chromosomes except chromosome 3, 8, and 22. When we analyzed the data from the three sets of hybrids made between human fibroblasts and mouse cells, there was a strong correlation between the presence of human chromosome 3 and the ability to repair UV-induced DNA and the loss of chromosome 3 and the loss of repair capacity. These data suggest that a gene or a series of genes required for DNA repair are located on human chromosome 3. When we analyzed the data from the other two sets of hybrids, the correlation between repair ability and chromosome 3 was not as clear. At least five clones were discordant for chromosome 3 and repair

ability. The contractor is investigating the possible reasons for the discrepancy between the data derived from fibroblast hybrids and hybrids made with lymphoblastoid cells or unstimulated lymphocytes.

During the course of these studies, an important question arose. Do the mouse input cells contribute to the level of DNA repair observed? One way to answer this question was to determine whether or not mouse input cells can complement defective DNA repair in cells from xeroderma pigmentosum patients (XP), a disease characterized by defective ability to repair UV-induced DNA damage. This disorder is genetically heterogeneous and at least seven different complementation groups have been described. Human x mouse somatic cell hybrids were isolated from fusion experiments between mouse cells and XP cells derived from five of the complementation groups (A-E). The results demonstrated that the mouse cells will complement defective repair capacity in four of the complementation groups (A-D), but not in a fifth group, Group E. These data suggest that the genetic defect in the complementation groups A-D is different from that found in Group E. These preliminary experiments also suggest that hybrid cells formed between the mouse and various complementation groups of XP provide a new and valuable probe for genetic and DNA repair analysis of the defective DNA repair seen in XP. These data further indicate that in man x mouse cell hybrids (employing human cells normal for excision repair capacity), the mouse parental cell can contribute one or more steps in the excision repair pathway. These data must be taken into account when interpreting the results of the gene mapping experiments.

Preliminary experiments were carried out to determine whether this complementation of defective repair in XP cells by mouse cells is biologically significant. This was done by testing hybrid clones for resistance to UV irradiation. XP cells are highly sensitive to UV irradiation. In the initial experiments, viable XP x mouse hybrid clones were isolated following UV irradiation. The defective DNA repair capacity of the XP cells had been complemented by the mouse cells. If these results can be confirmed, the data would indicate that defective repair of UV-radiation induced DNA damage can be corrected both biochemically and functionally.

Significance to Biomedical Research and the Program of the Institute:
The importance of these studies lies in:

1. The fact that most organisms, including man, possess several complex DNA repair systems.

2. The demonstrated association between defective DNA repair, cancer proneness, and increased sensitivity to physical and chemical environmental mutagens and carcinogens.

3. The need to elucidate the genetic basis of this polygenic system in order to understand the interactions of the numerous repair enzymes.

4. The fact that a knowledge of the chromosomal assignment of the genes required for DNA repair and the gene or genes defective in XP will be extremely useful in prenatal diagnosis and genetic counseling.

Thus, the potential importance of this project is that it will yield information on the genetic structure of the DNA repair mechanisms in man. This information is essential if we are to fully understand the functional relationships between DNA repair, mutagenesis and carcinogenesis.

Proposed Course: To continue these experiments utilizing human x mouse somatic cell hybrids to determine the number and kinds of genes required for the repair of UV-induced damage in man as well as the chromosomal assignment of these genes. This system will serve as a model for the genetic analysis of human repair mechanisms involved in repairing DNA damage induced by other agents.

Date Contract Initiated: September 17, 1979

Current Annual Level: $79,560

DEPARTMENT OF ENERGY/OAK RIDGE NATIONAL LABORATORY (YO1-CP-90211) (Formerly YO1-CP-50200)

Title: Respiratory Carcinogenesis - Dynamics of Neoplastic Development in the Respiratoiry System

Contractor's Project Director: Dr. Margaret Terzaghi

Project Officer (NCI): Dr. Paul Okano

Objectives: To develop in vivo and in vitro techniques for studying the development of respiratory tract cancers in vivo. To identify cellular changes occurring early during neoplastic development in carcinogen-exposed tracheal epitelium. To develop models for studying the effects of x-radiation and promoters on the dynamics of neoplastic development in tracheal mucosa.

Major Findings:

1. The effect of carcinogen dose on the dynamics of neoplastic development in DMBA-exposed tracheal epithelium.

    a. 50 µg DMBA yields 1/25 tracheal carcinomas with a latency period of 17 months, 350 µg DMBA yielded 33/36 tracheal carcinoma with latency periods of 5-18 months.

    b. At all carcinogen doses, except the lowest (5 µg), the EF frequency/trachea is stable with increased time post exposure. By one year following 5 µg EF, frequency decreased from 4 to <0.5 EF/10^4 cells and the growth rate of individual foci was also decreased.

    c. There is a marked time and dose effect on the frequency of agarose-positive EF. The higher the carcinogen dose, the earlier following exposure a significant increase could be detected.

2. Initiation-promotion studies, exposure of intact tracheal grafts.

    a. No effect of TPA on the frequency of EF or subculturability of EF isolated up to one year post-exposure to 50 µg DMBA was noted.

    b. The capacity of cholesterol alone to yield EF in vitro does not seem to be related to possible mutagens present in heated cholesterol.

c. Stearyl alcohol plus carbon has been found to be a good vehicle for future studies.

d. A marked effect of TPA on tumor induction in vivo by 50 µg DMBA was observed.

3. Initiation-promotion studies, exposure of cell populations isolated in vitro following "initiation."

a. Three populations with different morphologies have been inoculated into tracheal grafts.

b. Exposure to TPA has been initiated.

4. The effect of radiation on neoplastic development in cell populations with altered in vitro growth potential.

a. The same lines selected for the promotion studies above have been inoculated into tracheal grafts.

b. Cells have been irradiated with 400 rads x-ray.

c. No altered growth potential in vitro has been detected following irradiation of normal rat tracheal mucosa.

Significance to Biomedical Research and the Program of the Institute:
Bronchogenic carcinoma, the prevalent neoplasm of the respiratory tract in man, most likely develops as a result of chronic exposure to various carcinogens and other agents present in the environment which act as co-factors in the carcinogenic process. The increased susceptibility to carcinogens or co-carcinogens of individuals already exposed to subcarcinogenic doses of one or more agents is clearly of practical importance.

The EF assay in vitro, developed in this laboratory, allows the quantitating of the emergence of carcinogen altered and neoplastic cell populations in tracheal epithelium following exposure in vivo to varied doses of one or more carcinogens and co-factors, delivered at controlled dose rates. Using this in vivo-in vitro model, the effect of dose rate and co-factors on the dynamics of neoplastic development in vivo in respiratory tract tissues can be effectively studied. It is expected that these studies will help define those environmental factors which are of primary importance in the pathogenesis of respiratory neoplasms.

Proposed Course:
1. To write up the carcinogen-dose study for publication.

2. To complete collection of in vitro data from the promotion study.

3. To begin a small promotion study using the vehicle stearyl alcohol for carcinogen delivery.

4. To evaluate in vivo and in vitro the emergence of neoplastic populations in tracheas repopulated with various cell lines exposed to TPA or radiation.

Date Contract Initiated: September 30, 1979

Current Annual Level: 0

ILLINOIS, UNIVERSITY OF (NO1-CP-95631) (Formerly Thomas Jefferson University, NO1-CP-85673)

Title: Selective Inhibition of RNA Polymerase II Activity as a Diagnostic Tool to Detect Potential Carcinogens

Contractor's Project Director: Dr. Fu-Li Yu

Project Officer (NCI): Dr. Paul Okano

Objectives: To study the underlying molecular mechanisms for the selective inhibition of hepatic nucleolar (ribosomal) RNA synthesis, and the inhibition of the enzyme activity of RNA polymerase II by the carcinogens N-OH-2-acetylaminofluorene, aflatoxin B_1 and actinomycin D.

Major Findings: As observed in earlier experiments, two hours after aflatoxin B_1 injection (0.3 mg/100 g body weight), rat liver nucleolar RNA synthesis, in vitro, was inhibited by about 90%. In analyzing the mechanism of this inhibition, previous experiments had ruled out the possibility that the enzyme, RNA polymerase I, is directly inhibited by the carcinogen. The next possibility that impaired nucleolar DNA template function might be the cause for the observed aflatoxin B_1 inhibition of rat liver nucleolar RNA synthesis was then explored. When total nucleolar DNAs from both control and aflatoxin B_1 treated groups are isolated and compared for template efficiencies in directing RNA synthesis with solubilized RNA polymerase I from the control group, no difference is found. When nucleolar chromatin function was analyzed, however, it was found that aflatoxin B_1 treatment resulted in a dramatic reduction in the RNA chain length to only 13% of the control size. The question of whether the site of aflatoxin B_1 attack is on the ribosomal DNA template or on the regulatory nucleolar chromosomal proteins, remains to be answered. The decrease in RNA chain length after aflatoxin B_1 treatment may be a consequence of either a decreased elongation rate for nucleolar RNA synthesis or an increased RNase activity. Since RNase activity is not increased by aflatoxin B_1 treatment, the dramatic decrease in RNA chain length is believed to be a result of aflatoxin B_1 inhibition of the rate of RNA chain elongation.

It is a well-known phenomenon that when in vitro RNA synthesis in isolated rat liver nuclei and nucleoli are compared, the rate of nucleolar RNA synthesis is found to be more than 10 times higher. Basically, this can be a result of either the more efficient transcription of the nucleolar chromatin template or a higher concentration of transcribing RNA polymerase I enzyme molecules. Experiments have shown that the nucleolus, on a per-unit-weight of DNA basis, has a 10-fold higher RNA polymerase concentration than the nucleus, suggesting that it is the RNA polymerase I concentration rather than the nucleolar DNA template efficiency that is responsible for the observed high rate of nucleolar transcription under normal steady-state conditions.

E. coli RNA polymerase has been used widely in mammalian chromatin transcription. There are indications that artifacts may be generated by the use of this heterologous transcription system. E. coli RNA polymerase, when added to the

isolated nucleoli, can transcribe the nucleolar chromatin with good efficiency. The question is whether it transcribes the active or inactive regions of nucleolar chromatin. The ability of this bacterial enzyme to transcribe the nucleolar chromatin was found not to be affected by the following conditions:

1. The engaged RNA polymerase I is occupying the active regions of the nucleolar template.

2. The active nucleolar chromatin template function is blocked by aflatoxin B_1 treatment.

3. The active nucleolar chromatin is destroyed by DNase I digestion. Based on these findings, it was concluded that E. coli RNA polymerase transcribes nucleolar chromatin at regions different from that of the engaged RNA polymerase I, and that it transcribes the inactive regions of the nucleolar chromatin.

Significance to Biomedical Research and the Program of the Institute:
It is generally agreed that in cancer cells the mechanism of gene regulation is aberrant. When considering the possible mechanisms of chemical carcinogenesis, there are two distinctive, alternatives:

1. The ultimate carcinogen interacts with the DNA and the critical genetic information transfer is blocked or altered, consequently the cell becomes cancerous.

2. Alternatively, the ultimate carcinogen may interact with the DNA-dependent RNA polymerases, the enzymes that transcribe the genetic information directly from the DNA template for normal cellular function, and as a result, the enzymes may malfunction and produce infidel transcripts from an otherwise normal DNA template. When translated into proteins, the abnormal proteins (e.g., defective DNA polymerase, defective repressor proteins, etc.) may cause permanent transformation of the cell.

Recent studies in this laboratory have shown that the administration of aflatoxin B_1, a natural occurring potent liver carcinogen to rats, rapidly and profoundly inhibits both nucleolar RNA synthesis and the enzyme activity of RNA polymerase II, per se. The same findings are observed with several other chemical carcinogens with unrelated structures, e.g., N-OH-acetylaminofluorene, actinomycin D, and methylazoxymethanol acetate. These findings are important because they suggest that the chemical carcinogens may act concurrently at multiple, critical biological target sites, and that chemical carcinogenesis may arise as a combined consequence of impairment from both genetic and epigenetic origin.

Proposed Course: This contract ended on December 30, 1980. Continued research on the mechanism of aflatoxin B_1 inhibition of nucleolar RNA synthesis is being supported by a recently awarded research grant.

Date Contract Initiated: July 1, 1979

Current Annual Level: 0

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (NO1-CP-55630)

Title: The Significance of Experimental Carcinogenesis Data to Man

Contractor's Project Directors: Dr. Helmut Bartsch
Dr. Ruggero Montesano

Project Officer (NCI): Dr. David G. Longfellow

Objectives:
1. To develop and understand criteria and parameters for the extrapolation of experimental carcinogenicity data to humans.

2. To validate and improve rapid screening tests which may have a use in selecting chemicals for in-depth investigat on and/or in predicting the carcinogenicity of environmental carcinogens.

Major Findings:
1. Oxidative benzo(a)pyrene (BP) metabolism was studied in 12,000 x g (S12) supernatant fractions of surgical lung specimens obtained from lung cancer patients undergoing surgical resection. Arylhydrocarbon (BP) hydroxylase (AHH) activity was determined in both normal and tumorous lung tissue specimens from the same patient. A more than 20-fold inter-individual variation in AHH activity was found in both the normal and tumorous lung tissue samples investigated. The apparent K_M of the pulmonary enzyme (1-2.5 x 10^{-4} M) was identical in tumorous and normal tissue. High-performance liquid chromatography (HPLC) of ethyl acetate-extractable BP metabolites from the normal lung tissue of 6 patients identified phenols, dihydrodiols and quinones of BP. The mean yields of these metabolites were very similar to those obtained in assays in the presence of lung S12 from BDVI rats, except that more trans-9,10-dihydro-9,10-dihydroxy-BP (BP-9,10-diol) was formed in the presence of human lung S12. The formation of 3-hydroxy-BP (3-HO-BP) and BP-9,10-diol in human lung specimens, however, varied by up to 7- and 13-fold, respectively. In normal lung specimens, the total amounts of 3-HO-BP and 9-HO-BP formed correlated positively with the total amounts of trans-7,8-dihydro-7,8-dihydroxy-BP (BP-7,8-diol), BP-9,10-diol and trans-4,5-dihydro-4,5-dihydroxy-BP (BP-4,5-diol) formed ($r = 0.83$; $p < 0.05$). A positive correlation was also found between the AHH activity in normal and tumorous tissue from all cancer patients ($r = 0.24$; $p < 0.05$). The ratio (R) of AHH activity in tumorous tissue to that in normal tissue from the same lung cancer patient was <1 in 73 patients, 1 in 2 patients, and >1 in 11. In 7 of 10 subjects who underwent surgical resection for suspected lung tumors, but in whom histological analysis revealed no malignant tissue, R was >1 for the AHH activity in the inflammatory tissue to that in normal tissue. The observation of consistently lower AHH activity in malignant lung tissue parallels findings in hyperplastic liver nodules induced in rats by hepatocarcinogens.

The frequency distribution of pulmonary AHH activity in normal and tumorous tissue from 105 lung cancer patients, with all types of tumors, was compatible with a unimodal distribution of the enzyme activity. In a subset of 43 patients with squamous-cell carcinoma, the same distribution was seen; in those patients, AHH activity in tumorous tissue correlated negatively ($r = -0.18$; $p > 0.10$) with the number of cigarettes smoked per day prior to surgery. No differences were observed between the mean values for AHH activity in cases of squamous-cell carcinoma and those of adenocarcinoma. No relationship was found between AHH activity in normal or tumor tissue and the age of patients. It remains to be investigated whether

differences in pulmonary AHH activity represent a host risk factor in persons who smoke.

2. 1,N^6-Ethenoadenine (εA) and 3,N^4-ethenocytosine (εC) are formed when electrophilic vinyl chloride (VC) metabolites, chloroethylene oxide (CEO) or chloroacetaldehyde (CAA) react with adenine and cytosine residues in DNA. These adducts were assayed for their miscoding properties in an in vitro system using Escherichia coli DNA polymerase 1 and synthetic templates prepared by reaction of poly(dA) and poly(dC) with increasing concentrations of CEO or CAA. Following the introduction of etheno groups, an increasing inhibition of DNA synthesis was observed. dGMP was misincorporated on CAA- or CEO-treated poly(dA) templates and dTMP was misincorporated on CAA- or CEO-treated poly(dC) templates, suggesting that εA and εC may miscode. The error rates augmented with the extent of reaction of CEO or CAA with the templates. Base-pairing models are proposed for the εA.G and εC.T pairs. The potentially miscoding properties of εA and εC may explain why metabolically-activated VC and its reactive metabolites specifically induce base-pair substitution mutations in Salmonella typhimurium. Promutagenic lesions may represent one of the initial steps in VC- or CEO-induced carcinogenesis.

3. Previous studies have shown that chronic treatment of rats with relatively low doses of NDMA results in an increased removal of O^6-methylguanine from liver DNA over that with a single dose of NDMA; however, no effect was observed on the other alkylated DNA bases, 7-methylguanine and 3-methyladenine. The increased removal of O^6-methylguanine was associated with induction of the enzymic DNA repair system(s). More recent studies, aiming at a further characterization of this phenomenon, are reported here.

A series of experiments was carried out to examine the efficiency of this induced DNA repair system with regard to time and to the number of O^6-methylguanine molecules. Previous studies have shown that the maximum level of induction was achieved with a dose of 2.0 mg/kg NDMA administered over a period of 3 weeks.

It has been shown that in liver DNA of pretreated rats 10 min after a dose of 2 mg/kg ^{14}C-NDMA the O^6/7-methylguanine ratio is 0.058, whereas in control rats it is 0.096. When various challenge doses of ^{14}C-NDMA (0.2, 2.0, and 20 mg/kg) are administered to rats pretreated with 2 mg/kg unlabelled NDMA, an increased removal of O^6-methylguanine is observed with 2.0 and 0.2 mg/kg, but not with 20 mg/kg.

These findings indicate that the increased enzymic activity induced by pretreatment with NDMA has a limited and finite capacity to cope with the removal of an increased amount of DNA damage, i.e., the removal of an increased number of O^6-methylguanine molecules. Above that level no differences are detected in liver DNA of pretreated or control rats in the rate of removal of O^6-methylguanine by the constitutive enzyme.

Although the presence of O^6-methylguanine in DNA cannot be considered in isolation, it appears to be a critical determinant in the initiation of carcinogenesis by NDMA. Thus, it appears important to examine whether the modulation of repair of this alkylation product in liver DNA following chronic treatment with NDMA at various dose schedules is reflected in the carcinogenic dose-response to NDMA in this organ.

4. In view of the extensive use of estrogenic hormones by the human population, either as therapeutic agents, or in the composition of the contraceptive pills, it is important to investigate more thoroughly the adverse biological effects of synthetic hormones. Diethylstilbestrol, ethynylestradiol, estradiol-17β, and estrone were chosen for these experiments. Evidence of carcinogenicity in rodents has been reported for each of these compounds, but so far, only a few studies have been carried out in vitro.

Because it has been shown that isolated liver cells in suspension are efficiently able to metabolize steroid hormones, these chemicals have been tested in V79 cells with a cell-mediated system using primary hepatocytes from male and female rats as the metabolic layer. The incubation in the presence of the chemical to be tested was carried out at concentrations ranging from 25 to 100 μM, for 48 hr before plating the V79 cells to score for mutagenicity or toxicity. In the absence of hepatocytes, the 4 estrogenic hormones were very toxic, but not mutagenic. The co-cultivation of V79 cells with primary hepatocytes decreased the toxic effect induced by the sex hormones, except in the case of ethynylestradiol. However, no mutation, determined as 8-azaguanine or ouabain-resistance, was induced under these conditions by any of the hormones tested.

The lack of mutagenic activity of these hormones in this assay has been confirmed by the use of primary liver cells that originated from a rat treated with Aroclor, an inducer of drug-metabolizing enzymes.

Significance to Biomedical Research and the Program of the Institute:
The studies on the role of metabolism and DNA repair processes in the organ- or species-specific carcinogenicity of various chemicals and on the validity and reproducibility of various short-term tests for the detection of chemical carcinogens, are aimed at gaining a better understanding of the basic biochemical mechanisms of carcinogenesis. This will permit a better use of test systems to detect environmental carcinogens and a better approach for extrapolating experimental data to humans.

Proposed Course: This contract terminated on December 31, 1980.

Date Contract Initiated: September 1, 1974

Current Annual Level: 0

JOHNS HOPKINS UNIVERSITY (NO1-CP-55713)

Title: Studies on Significance of Mutation in Carcinogenesis

Contractor's Project Director: Dr. Paul O. P. Ts'o

Project Officer (NCI): Dr. David G. Longfellow

Objectives:
1. To quantitatively characterize the entire neoplastic transformation process of the normal diploid Syrian hamster cells in culture. To correlate the alterations of the growth properties of cells in culture with their tumorigenicities in animals by a comprehensive approach based on statistical analysis. To utilize this methodology

for the characterization of the progression of the neoplastic transformation process in culture.

2. To develop a somatic mutation system from the same Syrian hamster embryo cells so that neoplastic transformation and somatic mutation of the same population can be investigated concommitantly. To investigate the basic relationship between neoplastic transformation and somatic mutation.

Major Findings:

1. A Role for Chromosomal Variability in Neoplastic Transformation -- After a successful study on the correlation among in vitro cellular growth properties and tumorigenicity, as well as a comprehensive characterization of neoplastic transformation, progress can now be made in the study of the basic mechanisms of neoplastic transformation. Previous findings indicate that point somatic mutation is not a sufficient model for neoplastic transformation, althrough a direct perturbation to DNA is sufficient to initiate the neoplastic transformation process, leading to tumorigenicity after a sufficiently long progression period. Much experimental evidence suggests that chromosomal variability or karyotypic abnormalities are related causally to neoplasia. Two approaches have been adopted to investigate this problem in detail. The first approach involves a careful study of pure populations of neoplastic cells. A comprehensive study of a clonal subdiploid tumor cell line derived from Syrian hamster embryo (SHE) cells revealed that extreme karyotypic variability is a characterization of these cells. It should be noted that these cells are extremely tumorigenic. Injection of 1-10 cells would induce tumors in newborn hamsters. Fusions of these tumorigenic cells with diploid SHE cells shows initial suppression of the anchorage independent phenotype, a trait closely related to tumorigenicity. However, rapid segregation of anchorage independent cells appears which approximates the rate of chromosomal loss in these highly unstable cells. These results are consistent with the notion of a recessive gene dose-dependent nature for the anchorage independent phenotype. The measured frequency of nondisjunction in these cells could permit conversion of the putative heterozygous preneoplastic cells into neoplastic anchorage independent homozygotes at the rate observed. These studies implicate chromosomal variability and random allelic assortment as a potential pathway in neoplastic progression. The second approach is to test this theory directly on human cells. A widely used antifungal drug, griseofulvin, has not been found to be mutagenic in bacteria tests but does affect microtubule assembly in mammalian cells leading to abnormal karyotypes. Therefore, griseofulvin may be used as an agent to specifically perturb chromosomal segregation in diploid human fibroblasts without inducing DNA/chromosome breakage. After the appropriate exposure of human cells to griseofulvin, aneuploid cells with chromosomal numbers ranging from 16-90 per cell were found in about 5-10% of the treated human cell populations. Thus, an effective procedure of producing aneuploid human cells without direct DNA damage can now be established. The significance of this induced aneuploid conversion of human cells in relation to recessive gene expression, cellular senescence, and neoplastic transformation, with or without pretreatments of mutagens/carcinogens, is under active investigation.

2. Neoplastic Transformation of SHE Cells and Adult Fibroblasts -- In the past, most of the studies on in vitro neoplastic transformation were done on rodent embryos. To understand the interrelationship among differentiation, escape from in vitro senescence and in vitro neoplastic transformation, these phenomena have been investigated, in fibroblast cultures established from 12-day old embryos, young (6 months) and old (20-26 months) adult Syrian hamsters. Adult hamster diploid skin fibroblast generally senesced about 10-15 population doublings (PD) in contrast to

embryonic fibroblasts which proliferated until 30-60 PDs. In general, embryonic diploid fibroblasts can be neoplastically transformed in vitro by exposure to carcinogens, and the acquisition of neoplastic phenotypes occurs progressively over 10-60 or more PDs. Preliminary investigations indicate that adult skin fibroblasts normally senesce unless exposed to carcinogens. Anchorage independent growth of these adult skin fibroblasts can be detected approximately 60 PDs after treatment. Currently, no quantitative comparison can yet be made, though preliminary results suggest that adult skin fibroblasts are more difficult to transform in terms of lower frequency and longer progression than embryonic fibroblasts. A more quantitative evaluation is needed, particularly so that the adult hamster skin fibroblasts can be studied in comparison to human fibroblasts, since the human fibroblasts used is the experiment usually are not at the embryonic stage.

Significance to Biomedical Research and the Program of the Institute:
The research described provides a direct test of the importance of chromosomal variability and induced karyotypic abnormality as the causes of neoplasia. It is important to develop an effective procedure for inducing aneuploidy in human cells without damaging the DNA. In the other experiments, neoplastic transformation can now be studied as a function of the developmental process. Such fundamental knowledge is especially useful for interspecies comparison between humans which are the subjects for risk assessment, and rodents, which are the subjects for biotesting.

Proposed Course: Syrian hamster cells have served very well as a model system for neoplastic transformation, and the knowledge from this system allows the scientific community to investigate the neoplastic transformation of human cells. Also, mechanisms of transformation can now be investigated much more critically. Results have been obtained on the specific effects of DNA breaks, and questions can now be asked about the specific effects of induced chromosomal variations (without DNA damage) as a cause of neoplastic transformation. It is also clear that the developmental process and stages exert a profound influence on neoplastic transformation. These problems certainly deserve major attention in the coming years. Contract ends August 30, 1981.

Date Contract Initiated: June 27, 1975

Current Annual Level: 0

JOHNS HOPKINS UNIVERSITY (NO1-CP-85670)

Title: Identification of Heterozygous Carriers of DNA Repair Defects

Contractor's Project Director: Dr. Lawrence Grossman

Project Officer (NCI): Dr. David G. Longfellow

Objectives: To immunologically assess the level, distribution, and nature of DNA repair enzymes in cell lines derived from patients with DNA repair deficiencies.

Major Findings: During the first two years, enzymes involved in the repair of DNA were isolated and purified from human placenta. Those xeroderma pigmentosum cell lines available from the Mutant Cell Repository in Camden and the American Type Culture Collection which constituted XP-A, XP-C, XP-D, XP-E, XP-F and variants are

currently being maintained on uniform growth media with samples of all passages stored in the frozen state. In addition, primary cell lines were established from skin biopsies of an XP-A heterozygote from the same family as the affected offspring and an XP-C homozygous patient and the proband's mother. In this way, the equivalent of isogenicity is maintained in all cell line pairs.

The antigenicity of the six enzymes that in sequence repair depurinated/depyrimidinated DNA has been studied leading to the production of antisera to four of the six. The 3'→5' specific exonuclease, DNase VII and the 5'→3' exonuclease DNase VIII are currently being isolated in quantities sufficient for antibody production.

One of the major stumbling blocks has been establishing sensitive immunoassays for either antibody or antigen levels. Much of the difficulty with the radioimmunoassay has been circumvented by the use of two techniques. The ELISA assay has been established, and its sensitivity is sufficient for all planned studies.

Techniques for radioimmunoassays on aminothiophenol paper (ATPH) will also permit evaluations of isoelectric points and molecular weight changes of mutant protein forms, because proteins can be electrophoresed from either SDS gels or ampholine plates onto the ATPH paper for the radioimmunoassays or ELISA assays.

Significance to Biomedical Research and the Program of the Institute:
During the next two years, the contractor should be able to determine the levels of six DNA repair enzymes, their distribution, and whether their primary structure has been affected by this disease.

Proposed Course: To determine the numbers of skin fibroblasts required to quantitatively assess the level of the apurinic/apyrimidinic endonuclease, DNase VII, DNase VIII, DNA polymerase β and α and polynucleotide ligase. The cellular distribution of each enzyme will be determined by the immunoperoxidase staining technique. The isoelectric points (pIs) and the molecular weights of the enzymes in native and denatured forms will be assayed by radioimmunoassays.

When the levels and distribution of these enzyme antigens can be standardized, a detailed comparative study will be made in xeroderma pigmentosum homozygous and heterozygous cell lines. Future directions include a study of cells derived from families with familial polyposis; complete characterization of affected enzyme structure and function is to be expected.

Date Contract Initiated: September 30, 1978

Current Annual Level: $218,393

MISSOURI, UNIVERSITY OF (NO1-CP-75946)

Title: Retroaldol Type Fragmentation of β-Hydroxy Nitrosamines which may be Environmental Carcinogens

Contractor's Project Director: Dr. Richard N. Loeppky

Project Officer (NCI): Dr. Larry Keefer

Objectives: Specifically, the contractor shall analyze trace quantities of nitrosamines which may be formed from N-nitrosodiethanolamine (NDELA) in metal-working fluids and to trace the fate of NDELA in these fluids by using ^{14}C-labeled NDELA. The objective of the workscope is as follows:

1. Develop methods to characterize trace nitrosamines in metal-working fluids containing NDELA.

2. Determine the fate of NDELA-^{14}C in metal-working fluids.

3. Synthesize and study the chemistry of nitrite esters related to diethanolamine.

4. Study the retro-aldol fragmentation of β-hydroxynitrosamines which may be present in environmental samples.

Major Findings: Much of the time during the current contract year was devoted to the synthesis of ^{14}C labeled N-nitrosodiethanolamine (NDELA), the development of analytical methods for the analysis of NDELA and its anticipated decomposition products in metal-working fluids, and the construction of a model grinding machine to determine the fate of NDELA during its inadvertant employment in metal-working fluids.

To date these experiments have shown that:

1. The developed analytical method can detect NDELA and other nitrosamines in metal-working fluids at the level of 11 ppb.

2. Actual metal grinding experiments induced the rapid formation of NDELA from diethanolamine but not triethanolamine.

3. After an initial rapid formation of NDELA in metal-working fluids during model grinding, its concentration increases slowly, and if it decreases or is converted to other nitrosamines, these processes are not detected without the use of radiolabels.

4. In the presence of strong base and small amounts of formaldehyde, NDELA is rapidly converted into 2-hydroxyethylvinylnitrosamine. Since metal-working fluids contain formaldehyde "donors" as bacteriocides and are alkaline, these experiments could have considerable practical significance as all tested vinylnitrosamines are among the most carcinogenic of all nitrosamines.

Significance to Biomedical Research and the Program of the Institute:
The analytical method developed for the NDELA analysis is already being used by the FDA and other groups for nitrosamine analysis in cosmetics and should help reduce the level of NDELA in commercial products. The finding that NDELA forms rapidly only from the secondary amine suggests that its removal from metal-working fluids should help prevent NDELA formation and exposure to workers in this field.

The finding that formaldehyde catalyzes the conversion of NDELA to a vinylnitrosamine argues against the use of "formaldehyde donor" bacteriocides in metal-working fluids.

Proposed Course: Contract ended December 31, 1980

Date Contract Initiated: September 30, 1977

Current Annual Level: 0

NORTH CAROLINA, UNIVERSITY OF (NO1-CP-55707)

Title: Development of Detailed Methods and Protocols for Carcinogenesis Screening Using Cell Culture Assays - Task V, Epithelial Cells

Contractor's Project Director: Dr. Joe W. Grisham

Project Officer (NCI): Dr. Paul Okano

Objectives:
1. To study the temporal relationship between carcinogen exposure and expression of early markers of transformation in liver epithelial cells using ultimate carcinogens or active carcinogen metabolites and noncarcinogenic analogs.

2. To compare at least one of the early markers with tumorigenicity on back transplantation into animals.

3. To continue to culture liver cells, and determine their responses to carcinogen treatment.

4. To examine a series of chemicals consisting of both carcinogens and noncarcinogens when there is sufficient evidence that one of the early markers can be used as a reliable indicator of the carcinogenic potential of a compound.

Major Findings: In previous research on this project, it was demonstrated that the transformation process in vitro in cultured liver epithelial cells consists of a slow step-wise sequence of cellular alterations, beginning with damage and presumably incomplete repair of DNA and ending when the cells become tumorigenic. A year or more may be required for tumorigenicity to be acquired after single exposure of liver epithelial cells in vitro to a carcinogen. Steps in the transformation process include resistance to the cytotoxicity of the carcinogen, increased growth rate, increased colony forming efficiency, alterations in colony morphology, modifications in the cell membrane, aneuploidy, ability to grow in soft agar, and tumorigenicity on back transplantation into animals.

During the past 12 months, an intensive study of the potential for treated cells to produce tumors on back transplantation into animals has been conducted. Both athymic nude mice and one-day old syngeneic rats (Fischer 344) have been used as host animals. These studies are still in progress and the results are, therefore, incomplete. However, it is apparent from the results available at this time that one-day old syngeneic rats provide better hosts for tumor growth than do athymic nude mice. Preliminary evidence from these and other studies indicate that treated cells with diploid numbers of chromosomes will neither grow in soft agar nor produce tumors on back transplantation. A study of the early karyotypic and chromosomal alterations has been initiated. These studies indicate that the most frequent initial karyotypic change is a loss of one chromosome, with a corresponding appearance of a large biarmed metacentric marker chromosome. These observations

suggest that the marker chromosome arises by the fusion of two submetacentric or acrocentric chromosomes. Banding studies to identify the fused chromosomes have been started. These studies have also shown that initial abnormalities in karyotypes are preceded and accompanied by a variety of breaks, deletions, and rearrangements. Subsequent to the loss of a single chromosome, the karyotypes shift from a diploid (hypodiploid) mode to a complex mixture of pseudodiploid and pseudotetraploid patterns.

In other studies, the ability of transforming liver epithelial cells to grow in calcium-poor medium (containing 0.02 mM calcium) and to tolerate the toxic environment of the chemical carbon tetrachloride has been assessed. The ability of cells to grow in calcium-poor medium precedes the ability to grow in soft agar. From these studies, it is apparent that the acquisition of the ability of cells to grow in calcium-poor medium develops gradually over several generations and is not an episodic phenomenon. Similarly, transforming cells progressively develop the ability to grow in a toxic environment containing 800 µg carbon tetrachloride / ml tissue culture medium. The cellular mechanisms underlying ability of transforming cells to proliferate in medium lacking calcium or containing carbon tetrachloride is not known.

Significance to Biomedical Research and the Program of the Institute:
Work on this contract has shown that neoplastic transformation of hepatic epithelial cells by chemicals in vitro is a slowly developing, prolonged process in which cells acquire progressive alterations in their phenotypic expression before becoming tumorigenic. The sequence of cellular events observed in vitro mimics the sequential changes that have been observed during experimental hepatocarcinogenesis in vivo. Because the in vitro situation can be manipulated at will, studies of neoplastic progression in vitro may allow the mechanism(s) of this important process to be ultimately understood.

Proposed Course: This contract terminated on January 29, 1981.

Date Contract Initiated: June 30, 1975

Current Annual Level: 0

SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH (NO1-CP-85665)

Title: Identification of Heterozygous Carriers of DNA Repair Defects

Contractor's Project Director: Dr. Raju S. K. Chaganti

Project Officer (NCI): Dr. David G. Longfellow

Objectives: The overall objective of this contract is to develop, using chromosome instability (chromosome breakage and sister chromatid exchange) as the endpoint, methods for the detection of heterozygous genotypes for the genes causing the autosomal recessive disorders: Fanconi anemia (FA), ataxia telangiectasia (AT), Bloom syndrome (BS), and xeroderma pigmentosum (XP). FA, AT, BS, and XP homozygotes have an increased predisposition to cancer.

Major Findings: Lymphoblastoid Cell Lines (LCLs) -- During this year, the methodology has been established for developing LCLs following EB virus infection. Fourteen lines from FA and AT homozygotes and AT heterozygotes have been established. These lines are extremely useful in heterozygote testing.

Fanconi Anemia (FA) -- The Contractor has continued to investigate the reliability of their method for prenatal detection of carriers. The identification of these individuals is based on the differential response of their cells to the clastogenic effect of diepoxybutane (DEB), compared with cells from patients who show some of the clinical features of FA (aplastic anemia, radial defects, etc.), or normal controls. Of twelve individuals with a possible diagnosis of FA referred to this laboratory for testing, five were positive and seven negative in studies of their peripheral blood lymphocytes. The results of studies of FA obligate heterozygotes were consistent with previous findings by this contractor of increased chromatid breaks and exchanges after treatment with a non-toxic concentration of DEB that had virtually no clastogenic effect on normal cells. Of twelve individuals at risk for being carriers, five were positive, five were negative, and two were inconclusive in lymphocyte studies. Similar methods have been used to study aminiotic fluid cells from three pregnancies at risk for FA; one was diagnosed as affected and two as normal. Chromosome studies have also been performed on cultured fibroblasts from a variety of tissus from two affected abortuses. The growth of these cells in culture is more vigorous than FA cells grown from skin biopsies from patients, and they will be useful for future studies aimed at further deliniation of the cellular defect in FA. In addition, cultured fibroblasts from four affected individuals and their parents have been studied. Results of all these studies in FA confirm the reliability of our method for prenatal and postnatal diagnosis and carrier detection.

Ataxia Telangiectasia (AT) -- Peripheral blood lymphocytes from six ataxia telangiectasia heterozygotes were tested for their susceptibility to DEB-induced chromosomal breakage. Breakage was elevated compared to normal controls, but confirmation of these findings awaits further study.

Two normal, five AT homozygous, and two AT heterozygous LCLs developed in Dr. George Klein's laboratory in Stockholm were stressed with 250r of X-ray in order to compare the chromosomal response (breakage) of heterozygous cells with those of normal cells and homozygous cells. The large number of chromosome preparations from this experiment will be evaluated during 1981.

Significance to Biomedical Research and the Program of the Institute: Chromosome instability possibly is a reflection of the defective DNA repair systems present or postulated in these disorders. By studying the cytogenetic responses of cells carrying these genes to mutagens that will elicit the DNA repair mechanisms, it is expected that insight into the nature of the defects themselves will be gained. In terms of the cancer program, methods for detection of carrier individuals will enable recognition of individuals at risk for cancer.

Proposed Course: During the next year, the focus will be on developing a test for carrier detection of XP and AT. The Contractor will challenge blood lymphocytes and skin fibroblasts of XP heterozygotes with DEB, 8-methoxypsoralen and UVA, and UVB, and study chromosome breakage and sister chromatid exchange in them compared to that exhibited by similarly challenged normal lymphocytes and fibroblasts. The AT heterozygous fibroblasts and LCLs will be challenged with X-ray and bleomycin, and

the chromosome breakage in them will be studied and compared to that exhibited by similarly challenged normal fibroblasts and LCLs.

Date Contract Initiated: September 30, 1978

Current Annual Level: $73,913

SOUTHERN CALIFORNIA, UNIVERSITY OF (NO1-CP-65831)

Title: Carcinogenesis In Vitro: Initiation and Promotion

Contractor's Project Director: Dr. Charles Heidelberger

Project Officer (NCI): Dr. Paul Okano

Objectives:
1. To develop and standardize an initiation and promotion system with C3H/10T1/2 cells as a potential prescreen for carcinogens and tumor promoters.

2. To develop and standardize a liver cell-mediated activation of various classes of chemical carcinogens to transform C3H/10T1/2 cells.

3. To develop methods for assay of syncarcinogenesis of C3H/10T1/2 cells by combinations of weak carcinogens.

Major Findings:
1. C3H/10T1/2 cells were induced to differentiate into muscle cells by treatment with 5-azacytidine, and the effects of tumor promoters, nonpromoters and inhibitors of tumor promotion on this induced differentiation were investigated. There was a good correlation between the tumor promoting activity of the compounds and their inhibitory action on differentiation except in the case of phenobarbital, which did not inhibit differentiation. When the cells were treated after induction of differentiation with TPA and simultaneously with an inhibitor of tumor promotion, the inhibitory action of TPA was not affected.

The morphology of C3H/10T1/2 cells was changed within 30 minutes following treatment with 0.1 µg/ml of TPA; they became smaller and refractile with long beady processes. Such changes were observed in both logarithmic and confluent cultures and lasted about 72 hours. The cells grew slowly in 1% fetal calf serum and under conditions that produced doubling times of 70 hours or greater, TPA, but not phorbol, reduced the doubling time to about 50 hours. Saturation densities were also increased by TPA in 1% fetal calf serum. The effects of various tumor promoters and inhibitors of tumor promotion on the 5-azacytidine-induced differentiation of C3H/10T1/2 mouse embryo fibroblasts into adipocytes have also been studied. However, the Contractor found no general relationship between stimulation or inhibition of adipocyte formation and promoting activity in these cells. In view of the diversity of these results, it is unjustified to use effects on differentiation in model systems as a means to screen for tumor promoters or to define mechanisms of tumor promotion.

2. A series of cloned epithelial cell lines from regenerating mouse liver have been developed that have the capacity to metabolize diverse classes of chemical carcinogens (3-methylcholanthrene, 2-acetylaminofluorene, dimethylnitrosamine and

aflatoxin B) to cytotoxic metabolites. These cells are being used as lethally irradiated feeder layers for cell-mediated activation of various classes of chemical carcinogens and mutagens with C3H/10T1/2 mouse embryo fibroblasts as indicator cells for transformation.

The induction of aryl hydrocarbon hydroxylase by 3-methylcholanthrene in the ML-8 cloned mouse regenerating liver epithelial cells is highly significant; thus, these cells retain this aspect of their liver derivation. They also operate the urea cycle. However, they do not retain their liver-like capacities for: glucagon induction of tyrosine amino transferase, secretion of albumin, production of alpha-fetoprotein, nor gamma-glutamyl transpeptidase activity.

ML-8 cells treated with aflatoxin B^1 eventually were capable of inducing adenocarcinomas in immunosuppressed syngeneic mice. Hence, they are capable of undergoing oncogenic transformation. However, the same phenomenon was observed in the controls, indicating that spontaneous transformation had also occurred. These experiments are being repeated and extended to determine whether this system can be made useful to study transformation of epithelial cells by chemical carcinogens.

Significance to Biomedical Research and the Program of the Institute:
The C3H/10T1/2 mouse embryo cell line that the Contractor developed is being validated as a short-term test for environmental carcinogens in many laboratories. Since these cells do not activate aromatic amines or nitrosamines, conditions for external liver-derived activating systems for mutagenesis and transformation of these cells are being optimized. C3H/10T1/2 cells are the only major transformation system in which tumor promotion has been demonstrated. Hence, it is being validated with tumor promoters of diverse chemical types as a short-term assay for tumor promoters.

Proposed Course:
This contract expires July 31, 1981.

Date Contract Initiated: September 1, 1976

Current Annual Level: 0

TEXAS, UNIVERSITY OF (M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (NO1-CP-85671)

Title: Isolation and Purification of Human Polycyclic Hydrocarbon Metabolizing Enzymes and the Production of Antisera to the Pure Enzymes

Contractor's Project Director: Dr. Marilyn S. Arnott

Project Officer (NCI): Dr. Paul Okano

Objectives:
1. Isolation and purification of three carcinogen metabolizing enzymes - glutathione-S-transferase (GST), phenolsulfotransferase (PST), and UDP-glucuronyltransferase (UDPGT) - from human autopsy liver.

2. Preparation of specific antisera to each purified enzyme.

Major Findings: During the past 12 months, two forms of GST have been purified to homogeneity from human liver. One of these has particularly high activity with styrene oxide as substrate, and may be important in polycyclic hydrocarbon detoxication. SDS gel electrophoresis indicates two subunits of 22,000 and 26,000 daltons. One to three other forms of GST (depending on the liver) have been partially purified. These have negligible activity toward styrene oxide, but are active with 1-chloro-2,4-dinitrobenzene. The purification procedure involves affinity chromatography of a 105,000 x g supernatant over glutathione-Sepharose, followed by fractionation over DEAE-Sephadex and CM-Sephadex. The method has been used successfully with material from five different livers, and seems to be generally applicable. Rabbit antisera production is ongoing and development of a radioimmunoassay is underway.

During the same period, what appears to be a single form of PST has been purified from three human livers. The enzyme has a Km for phenol of 30μM, for 3-hydroxybenzo(a)pyrene (3-OHBP) of 0.4μM, and for the sulfate donor, 3'-phosphoadenosine-5'-phosphosulfate, Km = 3.8μM. The purified enzyme can also transfer sulfate to N-hydroxy-2-acetylaminofluorene, p-nitrophenol, β-naphthol, dehydroepiandrosterone, 17β-estradiol, and dopamine. The purification procedure involves affinity chromatography of a 140,000 x g supernatant over Blue Sepharose, ammonium sulfate precipitation, chromatography over DE-52, hydroxyapatite, and Sephadex G-100, followed by affinity chromatography over ATP-agarose. SDS-gel electrophoresis indicates a single band of 35,000 daltons. The enzyme is present in human liver in low concentration, but enough material has now been obtained to begin antisera production.

The third enzyme, UDPGT, has been isolated to 90% purity. The human enzyme behaves differently from reported rat and rabbit enzymes. It is much more sensitive to detergent inactivation, behaves differently during ammonium sulfate precipitation, and does not chromatograph on DE-52 like those isolated from rat and rabbit liver. The purification method employed for the human enzyme include solubilization of microsomes in Lubrol, followed by fractionation with polyethylene glycol. The active fraction is applied to DE-52, CM-52, Sephadex G-100, and finally, to an affinity column of UDP-hexanolamine-Sepharose 4B. Purification of the enzyme is followed by conjugation of 3-OHBP, but activities toward p-nitrophenol, 4-methylumbelliferone and morphine copurify. SDS gel electrophoresis of the partially purified material from the affinity column reveals two major bands of 46,000 and 50,000 daltons constituting at least 90% of the protein, plus five minor bands of lower molecular weights.

Significance to Biomedical Research and the Program of the Institute:
The availability of human carcinogen metabolizing enzymes in purified form will:

1. Provide a means for examining in detail the metabolic activation and detoxication of polycyclic aromatic hydrocarbon carcinogens in humans.

2. Facilitate comparison of carcinogen metabolism between human and experimental animal systems, thus, aiding in animal-to-human extrapolation of carcinogenicity data.

3. Lead to sensitive assays for carcinogen metabolizing enzymes in human tissue.

4. Allow for the preparation of monospecific antibodies, opening the way for development of radioimmunoassays for key enzymes.

These achievements will contribute to the overall goals of the National Cancer Institute, as they should lead to reliable methods for assessing individual cancer risk in humans, based on their ability to metabolize carcinogens.

Proposed Course: During the coming year, development and use of radioimmunoassays for PST and the various forms of GST will be emphasized. In addition, the enzymes will be characterized in forms of amino acid composition molecular weight, subunit structure, and substrate specificity. The focus on UDPGT will be to improve the purification procedure in order to obtain homogeneous enzyme for use in antisera production, physicochemical characterization, and kinetic analyses.

Date Contract Initiated: September 30, 1978

Current Annual Level: $115,340

VANDERBILT UNIVERSITY MEDICAL CENTER (NO1-CP-85672)

Title: Isolation and Purification of Human Polycyclic Hydrocarbon Metabolizing Enzymes and the Production of Antisera to the Pure Enzymes

Contractor's Project Director: Dr. F. Peter Guengerich

Project Officer (NCI): Dr. Paul Okano

Objectives: The overall objective is to utilize purified carcinogen-metabolizing enzymes of human origin to better understand the roles of these enzymes in chemical carcinogenesis. Specific goals are:

1. Purification of NADPH-cytochrome P-450 reductase to homogeneity.

2. Purification of human liver microsomal epoxide hydrolase to homogeneity.

3. Purification of several of the individual human liver cytochromes P-450 to homogeneity.

4. Preparation of antibodies to each of the above purified enzymes.

5. Reconstitution of mixed-function oxidase activity towards several environmentally-important substrates.

6. Physical characterization of purified epoxide hydrolase, cytochromes P-450, and NADPH-cytochrome P-450 reductase.

7. Comparison of substrate specificities of human liver microsomal cytochromes P-450 with those of the enzymes obtained from laboratory animals.

Major Findings: During the contract period, the contractor accomplished the purification of the three enzymes in question, namely, cytochrome P-450, NADPH-cytochrome P-450 reductase, and epoxide hydrolase. These proteins all appear to be homogenous as judged by polyacrylamide gel electrophoresis under denaturing conditions. Antibodies have been raised to cytochrome P-450 and epoxide hydrolase in rabbits and antisera are specific, as judged by immunodiffusion precipitin

analysis and other techniques. Antisera to human NADPH-cytochrome P-450 reductase was not raised, because antibodies raised to the rat liver enzyme cross-react.

Recently, in collaboration with Dr. H. V. Gelboin of the NCI Intramural Program, antibodies were raised to human liver cytochrome P-450 and epoxide hydrolase in mice, and hybridoma lines producing monoclonal antibodies to the human liver proteins were developed. Initial studies suggest that these cell lines produce highly specific antibodies, although the titer of these antibodies has been lower than that obtained with rabbit antisera.

An immunoelectrophoretic technique for use with the human liver enzymes was also developed. Crude protein mixtures can be separated by electrophoresis in denaturing gels, and the proteins can be electrophoretically transferred to nitrocellulose sheets. Microsomal proteins seem to be uniformly transferred, and the transfer efficiency is constant for a given protein over a certain range of mass. Proteins bound to the nitrocellulose sheets can be visualized by sequential treatment with (rabbit) antibody raised to the protein of concern, (goat) anti-rabbit immunoglobulin G, horseradish peroxidase/(rabbit) horseradish peroxidase complex, and 3,3'diaminobenzidine/H_2O_2. Bands corresponding to a given antigen appear brown against a white background. This technique has been used with all three of the human enzymes to demonstrate antibody-antigen specificity. In addition, the technique can be used with mouse hybridoma antibodies when rabbit anti-mouse immunoglobulin G is included in the staining schedule. Preliminary results indicate that the highly specific bands can be quantitated on the order of 1 pmol microsomal cytochrome P-450.

In collaboration with Dr. Gelboin, it was also demonstrated that purified human liver microsomal epoxide hydrolase can inhibit the binding of metabolites of benzo(a)pyrene-7,8-dihydrodiol to DNA in cultured human cells. This observation has significance in the elucidation of the enzyme in the complicated overall scheme of metabolism of benzo(a)pyrene.

Finally, epoxide hydrolase from human liver cytosol was purified, as well as microsomes. The cytosolic enzyme has the same subunit molecular weight and cross-reacts immunologically with the microsomal enzyme. The cytosolic enzyme, like the microsomal enzyme, also has catalytic activity towards styrene-7,8-oxide and benzo(a)pyrene-4,5-oxide. While the cytosolic enzyme is present in pathologically normal liver biopsy samples, it is yet uncertain if the enzyme is normally in the cytosol or if this activity represents a population of the microsomal enzyme with weak affinity for the endoplasmic reticulum.

Significance to Biomedical Research and the Program of the Institute:
Polycyclic aromatic hydrocarbons are some of the most studied of the chemical carcinogens in the environment. The enzymes that metabolize these and other carcinogens have been studied in laboratory animals, but data for human systems is limited. Many of these carcinogens require activation by these enzymes to exert their effects; however, the same enzymes also appear to be capable of detoxifying some of these compounds. A need exists to establish the relevance of the animal studies to human systems. Availability of the pure enzymes and their specific antibodies would permit the examination of the roles of these enzymes in large human populations.

Proposed Course: The plans for the remaining seven months of the contract period can be outlined as follows:

1. Elucidation of the role of cytosolic human liver epoxide hydrolase.

2. Further purification of human P-450s.

3. Production of antibodies to human P-450 and use in the examination of multiplicity of P-450 in humans.

4. Development of optimal conditions for mixed-function oxidation of polycyclic hydrocarbons in reconstituted enzyme systems.

Date Contract Initiated: September 30, 1978

Current Annual Level: $59,818

WISCONSIN, UNIVERSITY OF (NO1-CP-85609)

Title: DNA Repair Studies in Cultured Hepatocytes

Contractor's Project Directors: Dr. Henry C. Pitot
Dr. Alphonse E. Sirica

Project Officer (NCI): Dr. David G. Longfellow

Objectives:
1. The development of a bioassay for carcinogenic agents utilizing the DNA repair system of primary adult hepatocyte cultures.

2. A study of the optimal requirements for the induction of unscheduled DNA synthesis in primary adult hepatocyte cultures of the rat, mouse, and hamster.

Major Findings: During the past year, the demonstrated importance of maintaining primary hepatocyte cultures in a complete medium including dexamethasone and glucagon has been clearly established as was reported last year. In addition, the system has been further refined by several interesting alterations. First, the extremely high backgrounds which had been reported earlier from this laboratory have now been almost totally eliminated by the isolation of nuclei from the cell cultures following unscheduled DNA synthesis using [methyl-^{3}H]thymidine in the presence of 10 mM hydroxyurea. After the nuclei are isolated from the cultures, the DNA is extracted from these nuclei and the radioactive label incorporated into the DNA determined by standard methodologies. The results suggest that a big proportion of radioactivity extracted from hepatocytes following TCA precipitation is not incorporated into nuclear DNA, but is likely to be the result of [methyl-^{3}H]-thymidine catabolism. Further characterization of the non-nuclear incorporated thymidine will be undertaken.

Secondly, and perhaps more important, the Contractor reported last fall that DNA repair by the procarcinogen, 2-acetylaminofluorene and the direct-acting carcinogen, methyl methanesulfonate, in primary adult hepatocyte cultures was markedly enhanced by the addition of 25 mM nicotinamide to the culture. This enhancement was in part related to the metabolism of the procarcinogen, 2-acetylaminofluorene, but was not

at all related to any metabolism of the direct acting carcinogen, methyl-methanesulfonate. Further studies have now demonstrated that not only nicotinamide but also isonicotinamide and a variety of other compounds, all of which have in common a capacity to inhibit the activity of poly (ADP ribose) polymerase (a nuclear enzyme tightly bound to chromatin) are effective. More recently, unscheduled DNA synthesis induced by ultraviolet light has also been stimulated two-to-three fold by inhibitors of the polymerase. A number of compounds have been tested, and their ability to enhance unscheduled DNA synthesis in the presence and absence of isonicotinamide has been examined. In several but by no means all cases, isonicotinamide did enhance the unscheduled DNA synthesis produced by several of the compounds, especially DMBA and 4-aminobiphenyl. On the other hand, a slight but significant level of unscheduled DNA synthesis induced by 10 mM levels of thiourea and ethionine were inhibited by the addition of isonicotinamide.

Finally, the hepatocyte/unscheduled DNA synthesis assay used in this laboratory allowed, for the first time, the demonstration of the genotoxicity of the common antihistaminic drug, methapyrilene hydrochloride, a compound which has tested negative in the Ames test, the transformation test by Pienta and in the hepatocyte/DNA repair test devised by Williams.

Significance to Biomedical Research and Program of the Institute:
The development of this system for the monitoring of potential environmental carcinogens by measuring induced DNA repair in cultured hepatocytes, offers promise of an additional screening procedure to be employed with such techniques as the Ames mutagenesis assay and sister chromatid exchange. The principal advantage of the hepatocyte system is that the enzymes required to metabolize the pro form of many chemical carcinogens are inherent within the cell system being utilized and, thus, do not have to be added; eliminating the potentiality of destruction of sensitive enzymatic machinery required in the metabolism of certain carcinogens. The system is relatively simple and more rapid than most other screening assays; the entire procedure taking about 48 to 72 hrs.

The increased sensitivity of the hepatocyte system through the isolation of nuclei and the utilization of inhibitors of poly (ADP ribose) polymerase potentially can make this system a useful adjunct to add to several other rapid bioassay procedures for screening for carcinogenicity. In this way, this study will clearly foster the program of the National Cancer Institute aimed at the development and use of more exact and rapid screening assays for the determination of potentially dangerous compounds in our environment.

Proposed Course: Since the present contract ended in March, 1981, further work on the bioassay system will not be continued. However, with other funds available, the Contractor plans to investigate the mechanism of the enhancing effect on unscheduled DNA synthesis of inhibitors of poly (ADP ribose) polymerase.

Date Contract Initiated: March 29, 1978

Current Annual Level: 0

SUMMARY REPORT
RESEARCH RESOURCES

The Chemical Research Resources program of the Chemical and Physical Carcinogenesis Branch provides chemical standards and certain rodents of research interest to the carcinogenesis research community at large. Through a number of resource contracts the program has chemical carcinogen reference standards prepared, analyzed and distributed to cancer researchers around the world. Labeled forms of retinoids which have shown promise in studies conducted for the Chemoprevention Program are made available for pharmacologic and metabolic investigations. Rodents of interest for carcinogen testing and chemoprevention studies are produced according to NCI specifications and are made available to NCI contractors to assure a constant, reliable supply of animals as economically as possible. NCI grantees are also eligible to purchase these animals through their grants. Another resource contractor produces a strain of aged rats for use in the study of spontaneous prostatic carcinoma. The program also manages instrument loan arrangements which provide 11 NCI-owned thermal energy analyzers to research laboratories around the world for studies on the environmental occurrence and relevance of nitrosamines. This program is scheduled for recompetition in 1981.

Chemical syntheses for the program are provided by six contractors, four of whom provide chemical carcinogens and their derivatives for the Chemical Carcinogen Reference Standard Repository at IITRI (NO1-CP-05612). The other two contractors provide retinoids for the Chemoprevention Program.

At the Midwest Research Institute (NO1-CP-05613) a wide spectrum of derivatives of polynuclear aromatic hydrocarbons (PAH) are synthesized and purified. These derivatives, both nonlabeled and labeled (^{3}H, ^{14}C), are prepared by unequivocal methods to produce adequate quantities of well-characterized compounds of high purity ($\geq$ 98%) for distribution as metabolite standards through the NCI Chemical Carcinogen Reference Standard Repository. During the last year 24 new PAH derivatives were added to the inventory. This contractor also serves as the Radiochemical Repository for the program. From an inventory of 34 compounds nearly 250 samples have been sent to 134 authorized investigators in the United States, Japan, England, France, Canada, Sweden, and Germany during the last year.

Companion contract efforts at MRI (NO1-CP-05719) and at SRI International (NO1-CP-05614) provide for the resynthesis of PAH derivatives in order to maintain the inventory at the Repository. Once an unequivocal route has been developed and tested several times by the previously mentioned contractor, then these two contractors provide a continuing supply. Each contractor has specific parent PAH compounds for which he is responsible for providing derivatives. A second objective for these contractors is the syntheses of compounds from other classes that are needed in the Repository. Nitrosamines, aromatic amines, additional parent polynuclear aromatic hydrocarbons, aflatoxins, steroid derivatives, and physiologically active natural products are among the chemical classes made.

Another contract effort at SRI International (NO1-CP-85612) is for the synthesis and purification of heterocyclic analogs of polycyclic aromatic hydrocarbons. A series of nitrogen, sulfur, and oxygen heteroanalogs are being prepared that are either known to be constituents of environmental pollution or are analogs of known PAHs. The availability of these compounds should be an aid in assessing the potential harm to man, animals, and the environment resulting from airborne emissions from power plants that will be burning increasing amounts of coal.

Compounds provided by the above four contractors are distributed as reference standards by the Chemical Carcinogen Reference Standard Repository operated by ITT Research Institute (NO1-CP-05612). About 2000 samples were distributed to researchers who requested them during 1980. The inventory includes over 500 compounds derived from the synthesis contractors and from surplus stocks of the NCI Bioassay Program (now through the National Toxicology Program). This contract allows the NCI to provide compounds for pertinent experiments in Chemical Carcinogenesis which could not be carried out otherwise. Carcinogenesis research has been greatly stimulated by the availability of authentic reference standards and/or substrates. This can be attested to by the volume of published accounts of research citing the NCI Chemical Carcinogen and Radiochemical Repositories (IITRI and MRI) as the source of standards. A recently completed survey of 380 scientists who had requested compounds, during an 18 month period, from the repositories, had a 50% response rate and collectively reported 546 publications resulting from the use of the compounds provided. The responses were made up of 79% domestic and 21% foreign recipients.

Retinoids for testing by Chemoprevention Program contractors are synthesized in kilogram quantities by the Southern Research Institute (NO1-CP-85616). Radiolabeled retinoids are prepared for those compounds which show chemopreventive efficacy in tests such as the tracheal organ culture system performed at IITRI (NO1-CP-05610). SRI International (NO1-CP-05601) prepares radiolabeled retinoids which are subsequently made available to the research community for use in metabolic and pharmacologic studies.

Contractors at the University of Georgia (NO1-CP-85663) and the Southwest Research Institute (NO1-CP-85601) have been preparing retinoids in encapsulated form for stabilization of the molecules for delivery into biological systems.

The Chemical Resources Program of the Branch contributes to a Division of Cancer Treatment contract with Charles River Breeding Laboratories (NO1-CM-77141) which operates a primary center for rodents raised in germfree biocontainment. Animals from this resource are provided for specific workscope requirements of CPCB contractors and can be purchased by NCI grantees through use of grant funds.

At Harlan Industries (NO1-CP-55647) an aged colony of ACI rats is maintained for use by researchers interested in the cause(s), prevention, and treatment of prostatic carcinoma. This animal model has been shown to yield spontaneous prostatic carcinoma by two years of age and virtually 100% incidence of tumors is found in animals at 30-32 months of age. These animals are now available, at no charge (except shipping charges) for studies on the etiology and pathogenesis of prostatic cancer. Inhibition of the development of these lesions may be attempted by dietary and endocrine manipulation and chemoprevention. The contract for support of this colony will terminate in August of 1981. Embryo freezing to preserve a breeding stock will be attempted.

Ten Thermal Energy Analyzers (TEA) have been placed under loan agreements in laboratories around the world. An eleventh instrument currently awaits assignment. The instruments, which are very sensitive, selective analyzers for N-nitrosamines, were developed in part under contract to NCI by the Thermo-Electron Corporation (Waltham, Mass.). The instruments were purchased in 1975 and a loan program initiated to stimulate research and collaboration on the environmental and occupational occurrence of N-nitroso compounds. The major emphasis has been on

determining the incidence of these compounds in products for human consumption. This has involved inter-laboratory comparisons of analysis of the various samples. The recipients involved have used these instruments with gas chromatographic and with high pressure liquid chromatographic equipment in making a variety of important discoveries concerning environmental distribution of N-nitroso compounds. These discoveries have included the detection of nitrosamines as normal constituents of human blood, as contaminants in beer, in flame retardants, hydraulic fluids, machine cutting and grinding fluids, in deionized water, and in the occupational environments of leather and rubber workers. The current location of the ten instruments is as follows: Oregon State University; U.S. Department of Agriculture, Agricultural Research Service; Laboratory of the Government Chemist, London, England; U.S. Food and Drug Administration; Deutsches Krebsforschungszentrum Institute fur Toxikologie und Chemotherapie, Heidelberg, West Germany; Tallinn Polytechnical Institute, Estonia, USSR; Massachusetts Institute of Technology; Eppley Institute; American Health Foundation; and the International Agency for Research on Cancer, Lyon France.

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GEORGIA, UNIVERSITY OF (NO1-CP-85663)

Title: Encapsulation of Retinoids for Administration in Laboratory Diets

Contractor's Project Director: Dr. James C. Price

Project Officer (NCI): Dr. Carl E. Smith

Objectives: Contract objectives are as follows:

1. Develop encapsulation procedures for retinoid compounds using the three-phase suspension method and gelatin as the encapsulating agent.

2. Perform bioavailability studies of retinoid compounds to obtain mean and individual plasma level-time profiles after a single oral dose of each retinoid of the following formulations:

   a. The reference dose in solution or suspension.

   b. The encapsulated retinoid in suspension.

   c. The encapsulated retinoid in a standard diet.

3. Determine the single dose blood levels using HPLC procedures.

4. Determine stabilities of encapsulated retinoids in standard rat feed under ambient environment conditions, including elevated temperatures.

Major Findings: A method for encapsulating retinoid compounds has been shown to provide protection from environmental influences so that stability is increased over unencapsulated material by a factor of 70 in some formulations. These encapsulated products are readily bioavailable when tested in rats. Tertiary butylhydroquinone has been shown to be more effective than butylated hydroxyanisole or butylated hydroxytoluene as an antioxidant for encapsulated retinyl acetate. A suspension dosage form of trans-retinoic acid has been developed which is less stable than the encapsulated material but which has bioavailability comparable to solution forms and is more stable than the solution form.

Serum level profiles of trans-retinoic acid in rats have indicated that there is a saturation or storage phenomenon which is dose dependent. In contrast, 13-cis-retinoic acid serum level profiles show very little of this effect.

Significance to Biomedical Research and the Program of the Institute:
Retinoid compounds must be protected from air, light, and moisture to prevent rapid deterioration. Encapsulation will allow long-term feeding studies to be conducted without fear of misleading or ambiguous results caused by the deterioration of the retinoids. Pharmacokinetic information about the retinoids will make it possible to better relate observed pharmacologic effects to the dose given. Improved assay procedures developed out of this research will facilitate future studies of the compounds.

Proposed Course: Future work on this contract will include the following:

1. Encapsulation of various retinoids.

2. Stability studies on the encapsulated retinoids.

3. Bioavailability studies on encapsulated retinoids.

4. Blood level studies of dosed retinoids.

Further work on improvement of assay procedures is anticipated and some modification of the encapsulation procedures may be necessary to accommodate retinoids of different physical characteristics. Some development of the dosage form to improve bioavailability of the retinoids is also anticipated. Contract will terminate September 29, 1981.

Date Contract Initiated: September 30, 1978

Current Annual Level: 0

HARLAN INDUSTRIES, INC. (NO1-CP-55647)

Title: Development of Colonies of Aged Rats

Contractor's Project Director: Mr. Hal P. Harlan

Project Officers (NCI): Dr. David G. Longfellow
Mr. Clarence Reeder

Objectives: The objective of this contract has been to breed and age inbred ACI rats, obtained originally from the Alton Ochsner Medical Foundation, as a potential model for naturally occurring prostatic carcinomas. Males of the species are placed in a barrier facility on a monthly basis for the purpose of aging.

Major Findings: The project has progressed well during the past year. As reported previously, 201 male virgin and breeder ACI's 24-41 months of age were necropsied and reviewed histologically for the genesis of prostate carcinoma. The results of this work have been published in Volume 43, No. 6, page 517, 1980 of Laboratory Investigation. In November, 1980, 203 male virgin and breeder Sprague-Dawley rats were submitted to E.P.L. Laboratories for necropsy and histopathological review of aging changes, hyperplasias, and other lesions. This work is still in progress. The Sprague-Dawley strain had been similarly bred and aged as a possible alternative to the ACI rat, however, it was found that the life span was not sufficiently long to permit utilization even as a control animal. No prostate carcinoma has been observed in these animals. The stock evidences severe mortality beginning at 22 months of age.

During this past year the Sprague-Dawley colony was closed out and most animals were utilized by researchers interested in the aging process.

The ACI strain still is being maintained according to the original protocol. All data clearly show that it has a longer life span than the Sprague-Dawley rat as well as a longer reproductive life. Two papers are to be published describing findings

on the longevity and reproductive life spans of these two strains (Drs. T. P. Cameron and C. P. Lattuada).

In the ACI rats no difference was found between breeder and virgin male rats with regards to morphology or incidence of prostatic lesions. This suggests that any difference in sexual activity and its secondary effects plays no role on the development of these lesions.

Significance to Biomedical Research and the Program of the Institute: To insure a continuing supply of aged ACI/segHAPBR rats to the research community for tumor and aging studies. This work has provided a model for studying the etiology and pathogenesis of naturally occurring prostatic cancers.

Proposed Course: To continue production of the ACI animals through the duration of the contract. During 1981 the ACI colony will be made available to interested investigators on a first-come, first-served basis. Contract is scheduled to end August 29, 1981.

Date Contract Initiated: June 30, 1975

Current Annual Level: 0

IIT RESEARCH INSTITUTE (NO1-CP-05610)

Title: Bioassay of Retinoid Activity by Tracheal Organ Culture System

Contractor's Project Director: Dr. Leonard J. Sniff

Project Officer (NCI): Dr. Carl E. Smith

Objectives: The objective of this project is the bioassay of new retinoid compounds by the tracheal organ culture assay. Hamster tracheal organ cultures provide an experimental assay system for determining whether new retinoids can alter epithelial cell differentiation. Under conditions of vitamin A deficiency, the tracheobronchial epithelium forms keratinized squamous metaplastic lesions but in the presence of active retinoids, the process of keratinization is reversed toward columnar ciliated and mucus secreting cells similar to those observed in vitamin A normal animals.

Major Findings: During the initial months of this contract, the conditions necessary to make operational the tracheal organ culture assay for initial evaluation of the biological activity of new retinoids were established. Tracheas from hamsters maintained on a vitamin A-deficient diet are maintained in organ culture for 3 to 10 days. The assay measures the biological activity of new retinoids at concentrations as low as 10^{-11} to 10^{-12} M. Structure-activity relationships of 11 new retinoids were studied in the tracheal organ culture assay system.

A total of 28 assays (one retinoid dose response per assay) were performed. The retinoids received from BASF Aktiengesellschaft, Germany, were more potent than the reference substance, all-trans-retinoic acid, in reversing keratinization caused by retinoid deficiency in tracheal organ culture. Retinoids synthesized by SRI International have also been evaluated in this testing program.

Significance of Biomedical Research and Program of the Institute:
Studies performed using the tracheal organ culture assay to measure the intrinsic ability of retinoids to control epithelial cell differentiation, provides significant predictive value for the potential use of a new retinoid for prevention of epithelial cancer. Results from these bioassays can provide information for animal studies to determine whether biologically active retinoids have prophylactic and therapeutic properties against a number of epithelial tumors.

Proposed Course: As they become available, newly synthesized retinoids will be evaluated for biological activity in tracheal organ culture system.

Date Contract Initiated: August 30, 1980

Current Annual Level: 0 (multi-year funded in FY80)

IIT RESEARCH INSTITUTE (NO1-CP-05612) (Formerly NO1-CP-55646)

Title: Chemical Carcinogen Standard Reference Repository

Contractor's Project Director: Dr. James N. Keith

Project Officer (NCI): Dr. David G. Longfellow

Objectives: To provide reference standards of carcinogens and related materials for safe storage, handling, and delivery to requestors.

Major Findings: The Chemical Repository has completed its fifth full year of operation. During 1980, about 2000 samples were distributed to laboratories designated by the Project Officer. The stocks have grown from 74 compounds at the initiation of the program to 506 as of the end of 1980. Many of these are chemicals transferred from surplus stocks of the the Bioassay Program and most of the rest are obtained from various NCI synthesis programs. During 1980, coded samples and safety data were supplied to laboratories participating in a study of the suitability of the mouse lymphoma assay in the Strain A mouse as a short-term in vivo assay for carcinogens. Lots of 75 carcinogens and noncarcinogens which had been previously tested in the chronic animal bioassay were tested.

Since the latter part of 1978, the Repository has been supplying analytical standards of several nitrosamines for use by laboratories participating in the USDA-FSQS Recognized Laboratory Program for Nitrosamine Analysis.

Numerous letter and telephone requests are answered on safe handling procedures and specific properties of chemicals. Visitors interested in safety procedures are given a tour of the IITRI repository facilities. Property data sheets supplied with the samples include physical and chemical properties, hazard information and recommendations for emergency action, and disposal.

Significance to Biomedical Research and the Program of the Institute:
Besides the need for effective coordination of information flow, the carcinogenesis research community has a pressing need for a centralized source of well-documented reference compounds. To this end, a chemical repository for the safe storage, repackaging, and distribution of samples for the Carcinogenesis Program has been established. This facility, designed and operated in conformance with OSHA and EPA

regulations and the DHHS guidelines, receives material from suppliers, repackages as required for the users, and ships samples with analytical documentation and safe handling protocols.

Proposed Course: The Repository has become a useful distribution center for chemicals not commercially available and performs a number of other service functions as well. Support activities will continue throughout the five-year contract.

Date Contract Initiated: September 30, 1980

Current Annual Level: $275,000

IIT RESEARCH INSTITUTE (NO1-CP-55646)

See the new contract award NO1-CP-05612

MIDWEST RESEARCH INSTITUTE (NO1-CP-05613) (Formerly NO1-CP-33387)

Title: Synthesis of Derivatives of Polynuclear Aromatic Hydrocarbons

Contractor's Project Director: Mr. James C. Wiley, Jr.

Project Officer (NCI): Dr. David G. Longfellow

Objectives: The major objective of this program is the synthesis and purification of NCI-selected nonlabeled and labeled (^{3}H, ^{14}C) polynuclear aromatic hydrocarbon (PAH) derivatives of the following types: phenols; quinones; epoxides; dihydrodiols; diolepoxides; alkyl and hydroxyalkyl substituted parent hydrocarbons; nitro-PAH derivatives, PAH-DNA adducts; and sulfate, glucuronide, and glutathione conjugates. These derivatives are prepared by unequivocal methods to produce adequate quantities of well-characterized compounds of high purity ($\geq$ 98% for distribution as metabolite standards through the NCI Chemical Carcinogen Reference Standard Repository. Activities in support of the NCI Repository include the initial synthesis, maintenance of inventory through resynthesis, and shipments of compounds to authorized recipients of isotopically labeled PAH metabolites from a Radiochemical Repository at Midwest Research Institute (MRI). In addition, selected polycyclic hydrocarbon derivatives are synthesized for the National Institute of Environmental Health Sciences via an interagency agreement with the NCI.

Major Findings: During the last year 24 polynuclear aromatic hydrocarbon derivatives have been synthesized, purified, characterized, and either shipped to the NCI Chemical Carcinogen Reference Standard Repository or, in the case of the isotopically labeled derivatives, placed in the Radiochemical Repository at MRI. These derivatives represent new additions and have included ^{14}C- and ^{3}H-labeled dihydrodiolepoxide enantiomers of benzo(a)pyrene (BP); nitro-derivatives of pyrene, perylene, and BP; non-K-region chrysene diolepoxides; ^{3}H-labeled and unlabeled oxiranylpyrene and pyrene epoxides; phenanthrene epoxide; tetrols and triols of BP; glucuronide conjugates of BP, benz(a)anthracene, and 7,12-dimethylbenz(a)anthracene; and glutathione conjugates of BP. The Radiochemical Repository, maintained and operated by MRI, has from its inventory of 34 compounds shipped 248 ^{3}H and ^{14}C-labeled PAH metabolite samples to 134 authorized investigators in the United States, Japan, England, France, Canada, Sweden, and Germany during the last year.

Significance to Biomedical Research and the Program of the Institute:
In order to understand the cause, and ultimately the prevention of cancer, a detailed understanding of the chemical and biological events at the molecular level will be required. Polycyclic aromatic hydrocarbons and their metabolites, many of which have been shown to be potent carcinogens, provide ideal systems for such studies. Since it is almost impossible for laboratories lacking specific synthetic facilities and experience to synthesize any one of these compounds for a particular metabolic study, the NCI through this contract, has been able to provide compounds for pertinent experiments in chemical carcinogenesis which could not have been carried out otherwise. As a result, carcinogenesis research has been greatly stimulated by the availability of authentic metabolite derivatives (labeled and unlabeled) for use as reference standards and/or substrates.

Proposed Course: Novel compounds will continue to be synthesized upon the request of the NCI. A major effort will be devoted to the synthesis, purification, and characterization of the following types of PAH derivatives:

1. Metabolites of parent PAH currently in the Repository (e.g., phenols, epoxides, quinones, dihydrodiols, triols, tetrols, dihydrodiolepoxides, derivatized methyl and nitro analogs and conjugates).

2. Potential metabolites of other classes of parent PAH not currently in the Repository with emphasis on classes of wide environmental distribution (e.g., indeno(1,2,3-c,d)pyrene).

3. Sulfate, glucuronide, and glutathione conjugates of selected PAH phenols, alcohols, dihydrodiols, quinones, and epoxides.

4. Resynthesis of expended PAH Repository derivatives, up to two to three times to provide for modifications and improvements of reaction procedures and optimization of yields of the original synthesis.

5. Radiolabeled (^{3}H, ^{14}C) and mass-labeled (^{2}H, ^{13}C) parent PAH, their metabolites, and derivatives for distribution through the Radiorepository located at MRI.

6. Tetrols and triols of selected PAH (e.g., benzo(a)pyrene and 7,12-dimethylbenz(a)anthracene) for use as standard markers of diolepoxide formation in biological systems.

7. Mono-, di-, and trimethyl derivatives and metabolites of a selected group of parent PAH (e.g., chrysene, phenanthrene, fluoranthrene, pyrene, dibenz(a,h)anthracene, benzo(a)pyrene, benzo(e)pyrene, and benz(a)anthracene).

8. PAH-DNA adducts of selected PAH derivatives (e.g., BP-7,8-diol-9,10-epoxide-$2NH_2$ guanine, BP-7,8-diol-9,10-epoxide-$2NH_2$ guanosine, and BP-7,8-diol-9,10-epoxide-phosphoesters) for use as standards in DNA-carcinogen binding studies.

9. Nitro PAH derivatives of selected PAH of wide environmental distribution with special emphasis on PAH that appear on the EPA priority pollutant list.

Most of these PAH derivatives will be prepared by the same or extensions of methods which MRI has used in preparation of over 150 unlabeled and labeled PAH metabolites and derivatives on a previous NCI contract. In some cases, new techniques and procedures will be employed. MRI is aware that as research in the field of

molecular carcinogenesis advances, specific objectives may be somewhat modified or even significantly changed to include new classes of PAH, or to restrict some classes of compounds now under consideration.

Date Contract Initiated: September 30, 1980

Current Annual Level: $498,680

MIDWEST RESEARCH INSTITUTE (NO1-CP-05719)

Title: Synthesis of Selected Chemical Carcinogens

Contractor's Project Director: Dr. J. Richard Heys

Project Officer (NCI): Dr. David G. Longfellow

Objectives: The major objective of this program is the resynthesis of NCI-selected derivatives of benzo(a)pyrene, 7,12-dimethylbenz(a)anthracene, and cyclopenta(c,d)pyrene for inventory maintenance of the NCI Chemical Carcinogen Reference Standard Repository. The syntheses of the selected derivatives use previously established, unambiguous methods to provide well-characterized compounds of high purity and include unlabeled as well as ^{3}H- and ^{14}C-labeled compounds. A second objective of this program is the syntheses of compounds from other chemical classes including aromatic amines, steroid derivatives, additional polynuclear aromatic hydrocarbons, and physiologically active natural products.

Major Findings: In the five months since initiation of this program, nine derivatives of benzo(a)pyrene have been prepared, characterized, and sent to the NCI Chemical Repository. These derivatives have included the 8-phenol, the 1,6-, 3,6-, and 4,5-quinones, the 4,5- and 7,8-trans-dihydrodiols, the syn- and anti-7,8-dihydrodiol-9,10-epoxides, and the 4,5-epoxide.

Significance to Biomedical Research and the Program of the Institute: This contract provides the National Cancer Institute with pertinent compounds for experiments in chemical carcinogenesis. In response to the goals of the program, the preparation of these compounds allows researchers lacking synthesis capabilities to have available authentic samples of substrates and probable metabolites for their investigations of chemical carcinogenesis.

Proposed Course: The synthesis of unlabeled and ^{3}H and ^{14}C-labeled metabolites and conjugates of benzo(a)pyrene, 7,12-dimethylbenz(a)anthracene, and cyclopenta(c,d)pyrene will assure a continuing supply to the NCI Chemical Carcinogen Reference Repository. The labeled physiologically active natural product capsaicin-1'-^{14}C will be prepared, and as determined by NCI, additional labeled and unlabeled syntheses may include aromatic amines, steroid derivatives, and other polynuclear aromatic hydrocarbons and physiologically active natural products.

Date Contract Initiated: September 30, 1980

Current Annual Level: $185,235

MIDWEST RESEARCH INSTITUTE (NO1-CP-33387)

See the new contract award NO1-CP-05613

MIDWEST RESEARCH INSTITUTE (NO1-CP-75911)

Title: Synthesis of Radioactive Retinoids for Metabolic and Pharmacologic Studies Relating to Prevention of Lung Cancer and Other Epithelial Cancers

Contractor's Project Director: Dr. Ping-Lu Chien

Project Officers (NCI): Dr. Anita B. Roberts
Dr. Carl E. Smith
Dr. Charles A. Frolik

Objectives: The objective of this program is to synthesize NCI-selected radioactive retinoids for use as tracers in metabolic or pharmacologic studies, both in vivo and in vitro. The program involves the preparation of different retinoids with modification of the ring, side chain, or terminus and variations in both the radioactive isotope (^{14}C or ^{3}H) and the position of label incorporation.

Major Findings: Studies on the synthesis of two metabolites of retinoids needed in the elucidation of metabolic pathways were carried out during the remaining contract period. Practical synthetic routes to these two compounds had been identified when the contract expired at the end of September 1980. Preliminary studies aimed at the synthesis of 4,4-difluoro substituted retinoic acid, in which one of the major metabolic sites is blocked, was also conducted.

Significance to Biomedical Research and the Program of the Institute: Radioactive (^{14}C and ^{3}H) retinoid acid derivatives synthesized as a result of this program have been successfully used by investigators at NCI for elucidation of the metabolic pathway(s) and biochemical transformations of retinoids in vivo and in vitro. The results of these studies should prove beneficial in the design of more effective retinoids for chemoprevention of cancers in epithelial tissues.

Proposed Course: Contract terminated October 29, 1980

Date Contract Initiated: September 30, 1977

Current Annual Level: 0

NEW ENGLAND NUCLEAR CORPORATION (NO1-CP-75937)

Title: Synthesis of Radioactive Retinoids for Metabolic and Pharmacologic Studies Relating to Prevention of Lung Cancer and Other Epithelial Cancers

Contractor's Project Director: Dr. David Ahern

Project Officer (NCI): Dr. Charles Frolik

Objectives: The objective of the project is to synthesize radiolabeled retinoids for pharmacological and metabolic investigations.

Significance to Biomedical Research and the Program of the Institute:
Studies on the mechanisms of action of retinoids having significant chemopreventive activity, and their pharmacokinetic and pharmacodynamic properties are of the highest importance in understanding the fundamental and practical aspects of inhibition of carcinogenesis by these compounds. Radiolabeled retinoids are almost indispensible for many of these investigators.

Major Findings: The main focus of the work during this year was concentrated on the preparation of all-trans-5,6-dihydroretinoic acid (-10-^{3}H).

An initial approach was the catalytic reduction of all-trans-retinoic acid (-10-^{3}H). A number of different catalysts and conditions were tried. The reduction was interrupted after the uptake of one equivalent of hydrogen and the crude products were analyzed by high pressure liquid chromatography. The reduction products proved to be a complex mixture of compounds in which it was not possible to isolate the 5,6-dihydroretinoic acid (-10-^{3}H). The reduction route was abandoned in favor of a second approach which would require the epoxidation of all-trans-retinoic acid (-10-^{3}H). This epoxidation is known to be selective for tetra-substituted double bonds and generated the 5,6-epoxide of all-trans-retinoic acid (-10-^{3}H) in good yield. The anticipated reactions would be to open the epoxide ring to give the alcohol, which after derivatization as the tosylate, would then be smoothly hydrogenalized to give the desired 5,6-dihydroretinoic acid (-10-^{3}H). Unfortunately, all attempts to hydrogenalize the tosylate resulted in the production of complex mixtures from which the desired product could not be isolated. The above method was abandoned in favor of the following synthetic procedure.

β-ionone was catalytically reduced to give the 5,6-dihydroionone which was condensed with cyanoacetic acid. The resulting cyano acid was thermally decarboxylated in the presence of tritiated water to give a product which contained a tritium atom in the α position to the cyano group. The labeled cyano compound was converted to an aldehyde and condensed with β-methylethylcrotonate to produce a crude mixture of 5,6-dihydroretinoates (-10-^{3}H). The three major components were isolated, purified and characterized by NMR. The products were shown to be a mixture of roughly equal amounts of the 9-cis, 11-cis and all-trans isomers of 5,6-dihydroretinoic acid (-10-^{3}H). Efforts to isolate the all-trans-5,6-dihydroretinoic acid (-10-^{3}H) in a radiochemically pure form were unsuccessful.

Date Contract Initiated: September 30, 1977

Current Annual Level: 0

SOUTHERN RESEARCH INSTITUTE (NO1-CP-85616)

Title: Synthesis of Kilogram Amounts of Retinoids for Long-Term Animal Studies

Contractor's Project Director: Dr. Y. Fulmer Shealy

Project Officer (NCI): Dr. Carl E. Smith

Objectives: The objectives of this contract are the synthesis and complete characterization of certain retinoids selected by the Natinal Cancer Institute. These retinoids are synthesized in large quantities for long-term evaluation in animals.

Major Findings: All-trans and 13-cis retinamides were synthesized for long-term studies of chemoprevention of cancer in animals and for toxicological evaluation in animals. (±)-All-trans-N-(2-hydroxypropyl)retinamide, all-trans-N-(3-hydroxypropyl)retinamide, (±)-all-trans-N-(2,3-dihydroxypropyl)retinamide, and all-trans-N-(4-(pivaloyloxy)phenyl)retinamide were synthesized in quantities ranging from 0.75-1.5 kg. More than 3 kg. of all-trans-N-(4-hydroxyphenyl)-retinamide was prepared, and additional specimens were characterized. All-trans-N-butylretinamide, 13-cis-N-ethylretinamide, 13-cis-N-(2-hydroxyethyl)retinamide, and 13-cis-N-(4-hydroxyphenyl)retinamide were synthesized in quantities ranging from 125 grams to 375 grams. All of the retinoids were fully characterized and were sent to six different investigators for the biological studies mentioned above. In addition, specimens of all-trans and 13-cis-N-tetrazolylretinamide were analyzed by several methods to determine their purity prior to biological evaluation.

Significance to Biomedical Research and the Program of the Institute: Agents that prevent carcinogenesis, or the progression of the carcinogenic process to full malignancy in epithelia, have enormous potential for the control of human cancer. Studies in animals and in organ cultures show that retinoids have this potential, but new retinoids with improved chemopreventive action, decreased toxicity, and more favorable pharmacokinetic properties are needed. To achieve these goals, long-term experiments in animals must be performed. This contract provides large amounts of retinoids selected by the National Cancer Institute for these long-term studies of the chemoprevention of cancer.

Proposed Course: Retinoids requested by the NCI for its program on the chemoprevention of cancer will be synthesized, fully characterized, and sent to investigators designated by the NCI.

Date Contract Initiated: September 30, 1978

Current Annual Level: 0

SOUTHWEST RESEARCH INSTITUTE (NO1-CP-85601)

Title: Encapsulation of Retinoids for Administration in Laboratory Diets

Contractor's Project Director: Dr. Donald J. Mangold

Project Officer (NCI): Dr. Carl E. Smith

Objectives: The objective of this program is the stabilization of certain synthetic retinoids by microencapsulation in food or pharmaceutical grade materials and a demonstration of bioavailability of the encapsulated retinoids using the rat as the animal model. The stability of the encapsulated retinoids is being determined at 25° and 55°C with and without light.

Major Findings: Based upon previously reported results of the determination of the optimum microsphere formulations for the protection of trans-retinyl acetate and trans-retinoic acid, samples of microspheres of two experimental retinoids, 2-retinylidene-5,5-dimethyl-1,3-cyclohexanedione (retinylidene dimedone) and N-(4-hydroxyphenyl)-all-trans-retinamide (4-HPR), have been prepared using the most promising formulations. A 150-g sample of retinylidene dimedone (6 percent by weight) in a gelatin matrix with Tenox 20 antioxidant and sodium benzoate

preservative was forwarded to an NCI contractor for further evaluation in animal studies. A sample of microspheres of the identical formulation admixed with rat feed, followed by aging of the mixture at room temperature in light, gave a significantly lower decomposition of retinoid as compared to a standard stabilized retinylidene dimedone formulation containing trioctanoin, Tenox 20, and tocopherol deposited from solvent on rat feed.

In the case of 4-HPR, microspheres have been prepared using the most promising formulations similar to those used for retinylidene dimedone. A sample of 4-HPR-containing microspheres admixed with rat feed has been also subjected to the light aging test and gave a much lower rate of decomposition as compared to the "stabilized" formulation of 4-HPR deposited from solvent on rat feed.

Further basic formulation studies, using trans-retinyl acetate as a model retinoid, have shown that 5 percent of activated charcoal in the microsphere will protect the retinoid significantly against light degradation.

Bioavailability studies using the promising microspheres of each retinoid, with the rat as the animal model, have indicated the retinylidene dimedone or 4-HPR to be as bioavailable as the neat retinoid deposited from solvent on rat feed. The uptake of the retinoid was measured in the mammary tissue of female rats after feeding (ad libitum) over a two-week period. Total retinoid was determined by the colorimetric trifluoroacetic acid method.

Significance to Biomedical Research and the Program of the Institute:
Retinoids, vitamin A analogs, have been shown to have some ability to prevent chemical carcinogenesis in certain epithelial tissues of animals; however, the natural retinoids cannot be readily used because of toxicity and limited tissue distribution at the high dietary amounts required. Recently, it has been shown that several synthetic retinoids have high acivity and less toxicity for the prevention of cancer in animals in the results of work conducted by the National Cancer Institute. In order to extensively study the effect of these synthetic retinoids by feeding in animal diets, the materials must be stabilized, since most retinoids must be protected from oxidation, light, heat, moisture, and bacterial decomposition.

With the availability of relatively stabilized retinylidene dimedone and 4-HPR in the form of microspheres, larger scale and simpler animal feeding studies can be undertaken by other investigators. The formulations found promising for stabilizing the retinoids investigated to date should be useful in the preparation of stabilized formulations of many other experimental retinoids as microspheres.

Proposed Course: Larger scale runs of microspheres containing the experimental retinoids will be conducted to prepare samples for forwarding to NCI for further evaluation in animal studies.

Investigation of the preparation of microspheres of other experimental retinoids will be undertaken as the retinoids are made available by NCI. Evaluation of the microspheres for stability will be conducted at 25°C (with light) and at 55°C (without light). The microspheres also will be evaluated for stability at room temperature (with light) in rat feed. Studies will continue to be conducted with the most promising formulations to determine that the bioavailability of the retinoids has not been affected by the encapsulation. Contract will end September 29, 1981.

Date Contract Initiated: September 30, 1978

Current Annual Level: 0

SRI INTERNATIONAL (NO1-CP-05601)

Title: Synthesis of Radiolabeled Retinoids for Metabolic and Pharmacologic Studies

Contractor's Project Director: Dr. Hans H. Kaegi

Project Officer (NCI): Dr. Carl E. Smith

Objectives: The objective of the research is to synthesize adequate quantities of radioisotopically labeled retinoid compounds. These are to be used as tracers in the investigation of the metabolic and pharmacologic action of retinoid compounds as anticancer agents.

Major Findings: The synthesis of 11-cis-retinal-11-^{3}H was completed. Retinal-11-^{3}H (all-trans) was irradiated, and the desired 11-cis isomer was isolated by HPLC in 13% yield.

Tritiated all-trans-N-4-hydroxy-retinamide has been prepared. Ionylidine-acetaldehyde was prepared and separated into pure cis- and trans-isomers by preparative HPLC. The pure aldehyde was then reduced with lithium borotritide to the corresponding alcohol-^{3}H. Reoxidation with manganese oxide afforded the respective α-tritio aldehydes. The aldehyde was condensed with triethyl 3-methyl-4-phosphonocrotonate to give all trans-ethyl retinoate. Saponification gave all-trans-retinoic-11-^{3}H acid, parts of which were converted into the wanted N-4-hydroxyphenyl anilide.

All-trans-retinal-11-^{3}H was prepared by saponification of all-trans-retinyl acetate with sodium methoxide in methanol, followed by purification on HPLC.

Work is progressing on the preparation of tritiated all-trans-retinoic acid of high specific activity (30-50 Ci/mmole) and of all-trans-retinoic acid labeled with carbon-14 in positions 11 and 12.

Significance to Biomedical Research and the Program of the Institute: Retinoid deficiency enhances the susceptibility of experimental animals to chemical carcinogenesis. The application of retinoids can reverse carcinogen-induced hyperplasia or lesions, but the mechanism of action of the retinoids is largely unknown. Mechanistic studies with radiolabeled retinoids may enable investigators to design more therapeutically useful retinoids suitable for clinical application in cancer prevention.

Proposed Course: The synthesis of all-trans-retinoic acid labeled with ^{14}C and tritiated at a high specific activity (30-50 Ci/mmole) will be carried out. Additional compounds will be prepared as requested by the Project Officer, and the stock of already prepared retinoids maintained.

Date Contract Initiated: September 30, 1980

Current Annual Level: $182,007

SRI INTERNATIONAL (NO1-CP-05614)

Title: Synthesis of Selected Chemical Carcinogens

Contractor's Project Director: Dr. Elmer J. Reist

Project Officer (NCI): Dr. David G. Longfellow

Objectives: To synthesize quantities of known and suspected carcinogenic materials and their metabolites. The major objective of this program is the resynthesis of NCI-selected derivatives of benz(a)anthracene, 3-methylcholanthrene, chrysene, dibenz(a,h)anthracene, dibenz(a,c)anthracene and benzo(e)pyrene for inventory maintenance of the NCI Chemical Carcinogen Reference Standard Repository. The synthesis of the selected derivatives uses previously established unambiguous methods to provide well-characterized-compounds of high purity and includes unlabeled as well as ^{3}H and ^{14}C-labeled compounds. A second objective of this program is the synthesis of compounds from other chemical classes including nitrosamines, aflatoxins and mercapto steroids.

Major Findings: During the first six months of this project the following compounds were synthesized and shipped to the NCI Chemical Carcinogen Reference Standard Repository: N-butyl-3-carboxypropylnitrosamine, N-butylcarboxymethyl-nitrosamine and N-butyl-2-oxopropylnitrosamine. Work is proceeding on the synthesis of benz(a)anthracene-trans-1,2-diol-3,4-epoxy (anti), benz(a)anthracene-trans-1,2-diol-3,4-epoxy (syn), and benz(a)anthracene-trans-3,4-diol-1,2-epoxy (syn).

Significance to Biomedical Research and the Program of the Institute:
It is believed that the majority of cancers that occur in man are caused by substances in the environment. By studying these substances and their mechanisms of action, information may be derived that will lead to a means of prevention of cancer in man.

Proposed Course: Additional known and suspected carcinogenic compounds will be prepared as needed by the NCI.

Date Contract Initiated: September 30, 1980

Current Annual Level: $164,108

SRI INTERNATIONAL (NO1-CP-85612)

Title: Syntheses of Hetero-Substituted Polyaromatic Hydrocarbons

Contractor's Project Director: Dr. Elmer J. Reist

Project Officer (NCI): Dr. David G. Longfellow

Objectives: To prepare a number of heterocyclic analogs of polycyclic aromatic hydrocarbons. Each compound is to be characterized by elemental analyses, melting or boiling points, UV, IR, NMR, TLC, and/or HPLC and GC/MS when needed. Analytical characterization is performed on each compound. The compounds and analytical reports are shipped to the NCI Carcinogenesis Chemical Reference Standard Repository.

Major Findings: During 1980, the following compounds were shipped to the the NCI Repository: 2-azachrysene, 4-azachrysene, and 7H-benzo(g)pyrido(3,2-a)carbazole. Synthetic methods to prepare 2-azabenz(a)anthracene, 7-methyl-2-azabenz(a)-anthracene, and 7,12-dimethyl-2-azabenz(a)anthracene have been developed.

Significance to Biomedical Research and the Program of the Institute: Many polycyclic aromatic hydrocarbons have demonstrated carcinogenic activity. Heteroanalogs have been identified as environmental constituents but the carcinogenic correlation is much sketchier. The availability of known pure hetero aromatic compounds should aid considerabley in the study of this problem.

Proposed Course: More hetero aromatic compounds will be synthesized such as aza benzo(a)pyrenes, pyrido carbazoles, pyreno thiophenes, etc. Contract will end September 29, 1981.

Date Contract Initiated: September 30, 1978

Current Annual Level: 0

SUMMARY REPORT
SPECIAL PROJECTS

Included under Special Projects are multidisciplinary and interdisciplinary projects as represented by program projects and analogous contracts. Also included are the development and characterization of cell culture systems relevant to carcinogenesis research and development of analytical methodology and technology for the detection of chemical carcinogens in complicated matrices.

The development and characterization of cell culture systems relevant to carcinogenesis has involved both animal and human tissues. At American Health Foundation (NO1-CP-75952), an experimental system involving a combination of in vitro organ culture (rat colon) and xenotransplantation is being developed as a potential model to delineate direct effects of suspected carcinogenic and modulating agents that are involved in colon cancer. Two contracts concern development of cell or organ culture from human tissues. One, at the University of North Carolina (NO1-CP-75956) has been attempting to establish both cell and organ cultures of human endometrial tissues with high frequencies and with persistance of selected organ and cell cultures in vitro for periods exceeding a year. Studies of metabolism and binding of benzo(a)pyrene in organ culture as functions of physiologic cycles of estrogens and progestins have shown that the state of estrogenization affects both parameters. The contractor's efforts for the final year concern attempts to malignantly transform human endometrial tissues by chemical treatments in culture. The second contract, Middlesex Hospital Medical School (NO1-CP-75955), has established human urothelium in organ culture, and is comparing metabolism of various carcinogens in both human bladder organ cultures and rat bladder organ cultures.

Investigators at the British Foods Manufacturing Industries Research Association (NO1-CP-43337) have completed work on development of a method for analysis of N-nitroso compounds from complex matrices. The method appears to be applicable to all types of N-nitroso compounds and can be used to differentiate such compounds from nitrite and nitrate but not from nitrolic acids and thionitrates. At the University of Hawaii (NO1-CP-75915), a workable analytic method has been developed for the determination of cycasin and macrozamin in muscle, kidney, and liver tissues.

The UV-photocarcinogenesis sensitivity of selected stocks and strains of non-haired mice is being compared at Temple University (NO1-CP-85603). The breeding efficiency and immunologic traits of these animals are being evaluated in an effort to develop a preferred test organism for photocarcinogenesis studies.

SPECIAL PROJECTS

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CONTRACT NARRATIVES

SPECIAL PROJECTS

AMERICAN HEALTH FOUNDATION (NO1-CP-75952)

Title: Studies on Colon Carcinogenesis in Organ Culture of Intestinal Mucosa

Contractor's Project Director: Dr. Gary M. Williams

Project Officer (NCI): Dr. Lea I. Sekely

Objectives: The four specific objectives of this contract are as follows:

1. To develop organ culture techniques that permit the prolonged maintenance of functional fragments of rodent colon.

2. To study, in these cultures, the effects of model carcinogens and factors believed to be related to the development of human colon cancer.

3. To develop transplant techniques for maintaining explants of colon.

4. To determine whether carcinogen-treated colon from organ culture or fragments from intact colon are capable of developing carcinoma following transplantation.

Major Findings: Using defined culture conditions, rodent colon organ cultures have been maintained for a period of 35 days. These cultures, upon in vitro exposure to colon selective carcinogens, show a specific response. Furthermore, after implantation of the cultured colon explants into mammary fat pads, the mucosal epithelium is viable for at least six months. During the last twelve-month period, studies were undertaken specifically to extend the observations relevant to Objectives 2 and 4. Specific experiments were performed to examine in vitro effects of organ selective carcinogens and also to evaluate the biological significance of morphological abnormalties that were seen after acute in vitro exposure of the target tissue to carcinogens.

Rat colon organ cultures were utililzed to examine the interaction of 1,2-dimethylhydrazine (DMH), N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and of dimethylnitrosamine (DMN) with cellular DNA. The organ cultures were exposed to the carcinogens (5×10^{-4}M, 4 hrs.), followed by radioactive thymdine (^{3}H-TdR, 10uci/ml, 20 hrs.). DNA was isolated from dissociated mucosal epithelial cells and appropriate aliquots of the samples were monitored for DNA-associated ^{3}H radioactivity. ^{3}H-TdR incorporation was markedly reduced by the colon specific procarcinogen DMH, and by the direct-acting agent (MNNG), but not by the liver carcinogen DMN. Long-term maintenance of these cultures showed a greater enhancement of thymdine labeling index in DMH-exposed cultures than in DMN exposed cultures. Similarly, a single exposure to MNNG resulted in a higher incidence of mitotically active cells 14 days later.

These results, therefore, demonstrate a degree of specificity of the response of target tissue to colon-selective carcinogens. Enhancement of the labeling index suggests a possible alteration in the proliferative pool as a result of a persistent alteration produced by the carcinogens.

In order to assess the biological significance of acute effects of MNNG on rat colon organ cultures, the carcinogen-exposed tissue fragments together with non-exposed control fragments were implanted contralaterally into mammary parenchyma-free fat pads of syngeneic female recipients. Three to four months after transplantation, the MNNG-exposed fragments showed greater survival than the controls. Furthermore, enhanced cellularity and hyperactive nature of the colonic mucosa was evident by the presence of multilayered cubiodal epithelium and numerous mucosal glands in MNNG-exposed implants.

Significance to Biomedical Research and the Program of the Institute:
The present experimental system that involves a combination of in vitro organ culture and xenotransplantation could be developed as a potential model to delineate direct effects of suspected carcinogenic and modulating agents that are involved in colon cancer.

Proposed Course: The contract ended January 31, 1981.

Date Contract Initiated: September 30, 1977

Current Annual Level: 0

BRITISH FOOD MANUFACTURING INDUSTRIES RESEARCH ASSOCIATION (NC1-CP-43337)

Title: Development and Application of Methods for N-Nitroso Compounds and Their Precursors in the Environment

Contractor's Project Director: Dr. Clifford L. Walters

Project Officer (NCI): Dr. Larry K. Keefer

Objectives:
1. To develop methods of determination of non-volatile N-nitroso compounds, including those not extractable from a biological matrix.

2. To differentiate these N-nitroso compounds from nitrite and nitrate and other potential interfering agents.

3. To apply these methods to samples of environmental interest.

Major Findings: A method has been developed for determining N-nitroso compounds as a group in an extract of a food or other matrix using a chemiluminescence analyzer. The method is applicable to all types of N-nitroso compounds including N-nitroso derivatives of secondary amines and amides, guanidines, urethanes and sulfonamides. It can be used to differentiate such compounds from nitrite and nitrate, and the great majority of compounds potentially formed from them in a biological matrix, such as the pseudonitrosites of unsaturated lipids, but with the exception of nitrolic acids and thionitrates, which release nitric oxide under the same conditions as N-nitroso compounds.

The method has been applied to rat diets treated and untreated with sodium nitrite and akin to those employed to evaluate any carcinogenic effect of nitrite itself. Even in the diets untreated with nitrite, however, low concentrations of nitrite of the order of tens of μg per kg were determined using a chemiluminescence analyzer

along with compounds responding as N-nitroso compounds at the mg kg^{-1} level. The concentrations of both N-nitroso compounds as a group and nitrate in the whole diets generally increased with nitrite concentration and period of storage at ambient temperatures.

Significance to Biomedical Research and the Program of the Institute:
The detection of concentrations of nitrite of the order of tens of μg per kg and of N-nitroso compounds as a group at the mg kg^{-1} level in diets, etc., represents a new observation of relevance to both experimental carcinogenesis and to analytical epidemiology. Thus, the procedure developed for the determination at low levels of nitrite and of N-nitroso compounds directly on a biological matrix without prior extraction provides an eminently suitable technique for the survey in foods and the environment in general of such potentially carcinogenic compounds and of their precursors.

Proposed Course: The contract ended November 29, 1980.

Date Contract Initiated: June 1, 1974

Current Annual Level: 0

HAWAII, UNIVERSITY OF (NO1-CP-75915)

Title: Cycasin and Macrozamin as Potential Environmental Carcinogens

Contractor's Project Director: Dr. Hiromu Matsumoto

Project Officer (NCI): Dr. Elizabeth K. Weisburger

Objectives:
1. To develop analytical procedures for the determination of cycasin and macrozamin in meat.

2. To determine the quantities of cycasin and macrozamin in meat derived from cattle which have foraged on cycad plants.

Major Findings: There is a possibililty that the naturally occurring glycosidic carcinogens, cycasin and macrozamin, are being introduced into the human food chain via meat from cattle which have ingested cycad plants. The cycad constituents, cycasin and macrozamin, have been known since 1962 to be carcinogenic. A workable analytical method for the determination of the compounds in meat samples has been developed. The compounds are hydrolyzed by bacterial β-glucosidse, when ingested by animals, and the common aglycone methylazoxymethanol (MAM), the proximate carcinogen, is released. MAM added to meat samples, in low and high quantities, was not recoverable. Apparently, the very labile compound reacts with water and decomposes too rapidly to allow its extraction from meat.

Sheep were given a feed containing cycasin and macrozamin to simulate cattle grazing on cycads. Residual, parts per million, levels of the compounds were found in the liver, kidney and muscle. The compounds were found in larger quantitites in the liver and kidney than in muscle No MAM was detectable.

Significance to Biomedical Research and the Program of the Institute:
There is a high incidence of liver cancer among the natives of Papua, New Guinea, where there is a practice among the natives to consume tissues from ailing stock, including those suspected of suffering from cycad intoxication. It is very likely that such ailing cattle will have large residual amounts of cycasin and macrozamin in their tissues, based on the results from the experiments with sheep fed those compounds. If some tissues from cattle suffering from cycad intoxication are obtained and the compounds are found in significant quantities in the tissues, the cause of the high liver cancer in Papua, New Guinea, could be linked to the known carcinogens; cycasin and macrozamin.

Proposed Course: The Veterinary Officer of Papua, New Guinea, is attempting to obtain some tissues of cows which are suspected of suffering from cycad intoxication. It is difficult to obtain such meat samples because as soon as an animal is noticed to be affected, it is slaughtered and consumed by the owner. The tissues will be analyzed for cycasin and macrozamin if and when samples are obtained. The contract will end August 28, 1981.

Date Contract Initiated: July 1, 1977

Current Annual Level: 0

THE MIDDLESEX HOSPITAL MEDICAL SCHOOL (NO1-CP-75955)

Title: Studies of Carcinogenesis in Human Tissues

Contractor's Project Director: Dr. Marian Hicks

Project Officer (NCI): Dr. Lea I. Sekely

Objectives:
1. To obtain viable normal tissue with intact urothelium supported by mesenchyme, from human bladders, normally by transurethral biopsy of patients being examined for suspected urinary tract disease, but occasionally from cystectomy specimens freshly removed in the course of treatment.

2. The maintenance of human bladder organ cultures.

   a. To establish long-term (2-4 months) in vitro organ cultures of normal human bladder explants, and to establish cell cultures in parallel if possible.

   b. To determine the parameters for normal growth of human urothelium in vitro in organ culture by comparison with parameters already established for rat bladder explants in vitro in rat and human bladder in vivo.

3. Carcinogenesis in vitro using human bladder organ cultures.

   a. To apply methods of experimental carcinogenesis already developed in vivo and in vitro for the rat, to the induction of carcinogenesis in human bladder explants cultured in vitro, and to study the changes in the parameters established in 2.b. above.

b. To adapt methods developed with other organ systems to follow the uptake and metabolism of labeled direct-acting carcinogens on the urothelium.

c. To investigate in human bladder explants the effect of carcinogens known to be organotropic for the bladder in the rat.

d. To investigate the effect of anti-carcinogens, e.g., 13-cis-retinoic acid, on neoplastic change in human bladder explants in vitro.

e. To investigate the effect on human urothelium of compounds known to be found in human urine which could have a direct carcinogenic or co-carcinogenic effect, e.g., saccharin and cyclamate.

f. To investigate the ability of human bladder to metabolise urine-borne compounds such as dimethylnitrosamine, which are potentially carcinogenic, but which in animals are normally metabolised to their active carcinogenic metabolites in the liver.

g. To perform xenotransplantation of both untreated and carcinogen-treated human bladder explants into immune suppressed animals or hamster cheek pouches, to confirm that malignant transformation has occurred in those cultures deemed to have neoplastic growth patterns and subcellular markers of malignancy.

Major Findings: The metabolism of the polycyclic hydrocarbon benzo(a)pyrene by human and rat organ cultures has been investigated. Hydroxylated and conjugated metabolites have been detected in the incubation media by high pressure liquid chromatography (hplc) of the organic solvent-extractable metabolites. Compounds that cochromatographed with 3-hydroxybenzo(a)pyrene, 9,10-dihydro-9,10-dihydroxybenzo(a)pyrene and the proximate carcinogen, 7,8-dihydro-7,8-dihydroxybenzo(a)pyrene were formed. The difference in the proportions of these metabolites found in rat and human culture media was attributed to the tendency of rat tissues to accumulate 9,10-dihydro-9,10-dihydroxybenzo(a)pyrene, presumably because this material is a poor substrate for glucuronyl transferase. Covalent binding of benzo(a)pyrene to DNA was detected in both rat and human bladder organ cultures to extents similar to those found by other investigators using human tissues such as bronchus and colon.

The rat and human bladder cultures also metabolized ^{14}C-2-acetyl-aminofluorene, a known bladder carcinogen for rodents, to produce 7-hydroxy-2-acetylaminofluorene, 9-hydroxy-2-acetylaminoflluorene, 2-aminofluorene and the proximate carcinogen, N-hydroxy-2-acetylaminofluorene. The proportions of the hydroxylated metabolites by comparison with the 2-aminofluorene were smaller in the rat cultures because of the greater ability of the rat urothelium to form glucuronic acid conjugates.

Both human and rat bladders, thus, possess enzymes capable of metabolizing two known carcinogens to their proximate carcinogenic derivatives, and in the case of benzo(a)pyrene this results in covalent binding to DNA. This implies that the metabolic potential of the urothelium is such that initiation of carcinogenesis may result from exposure to unmetabolized carcinogens in the urine.

Studies of the biological effects of methylnitrosourea (MNU) and benzo(a)pyrene (BP) on organ cultures of normal human and rat bladder have also been initiated. The

half-life of the nitrosamide MNU has been determined in Hank's balanced salt solution over a range of pH values, and the effects of one or two doses of 250 µg/ml or 500 µg/ml MNU within the first 14 days in vitro assessed histologically. No gross toxicity was found between 2 and 26 days after treatment. Single treatments with 500 µg/ml have been selected, and experiments involving up to 6 single doses are in progress.

Cultures from 16 patients treated with one or two doses of BP at 1,5, or 10 µg/ml for 24 hours have also been observed, and no toxicity found up to 28 days. Higher doses and increased numbers of fractions are now being studied.

A single dose of 500 µg/ml MNU on rat bladder organ cultures produced long-term toxic effects on the smooth muscle of the bladder wall. Pyknotic nuclei were observed two days after treatment, and by 14 days the smooth muscle wall was necrotic. By comparison with control cultures, at 11 days the MNU-treated cultures showed some loss of epithelial polarity, and by 14 days some were moderately dysplastic. By 28 days, all treated cultures showed dysplasia, and some in-growths of the surface epithelium into the underlying stroma occurred.

In preparation for tumorigenicity testing of carcinogen-treated human bladder organ cultures, a xenotransplantation model using neo-natally, thymectomised, x-irradiated mice protected with cytosine arabinoside is being investigated. Four established human bladder cell lines were shown to produce tumors in this host, but not in immunologically competent controls when 10 cells were implanted subcutaneously. All tumors were palpable within 10 days of xenografting. Tissue fragments from eight human primary transitional cell carcinomas have been implanted into these mice, and although they rapidly produced palpable nodules, these regressed within 14-30 days. In control mice, no tumor growth occurred and original implants disappeared by 14 days. Two early tumor nodules from immuno-suppressed mice transplanted to other immuno-suppressed recipients showed the same pattern of regression.

Significance to Biomedical Research and the Program of the Institute:
The in vitro human bladder model being developed by the contractor should provide an alternative or parallel system to experimental laboratory animals for assessing biological changes associated with the biogenesis of bladder cancer, and for investigating the biochemical action of known carcinogens on human bladder tissue.

Proposed Course: This contract terminates April 1, 1981. The investigators concluded their experiments on Task III as set out above.

Date Contract Initiated: July 30, 1977

Current Annual Level: $15,000

NEBRASKA, UNIVERSITY OF (EPPLEY INSTITUTE FOR RESEARCH IN CANCER AND ALLIED DISEASES) (NO1-CP-33278)

Title: Environmental Carcinogenesis Research and Bioassays

Contractor's Project Director: Dr. David B. Clayson

Project Officers (NCI): Dr. David G. Longfellow
Dr. Carl E. Smith

Objectives: The bioassay of suspected chemical carcinogens, definition of the biological and chemical mechanisms of the carcinogenesis process, and the analysis of trace levels of environmental carcinogens are three principal research aims under this contract.

Major Findings: Several long-term animal studies of carcinogenesis are in their final stages of completion. These studies are in the area of pancreatic and hormonal carcinogenesis. In both of these areas, final histopathologic analyses are underway. It is too early to present results at this time on these still incomplete pathologic analyses. Studies on the possible anticarcinogenesis of metamucil and α-tocopherol are under final stages of histo-pathological evaluation. Present results indicate that metamucil has no protective effect on dimethylhydrazine-induced colonic carcinogenesis.

Significance to Biomedical Research and the Program of the Institute:
The definition of the biochemical and chemical mechanisms of the carcinogenic process as well as the trace levels of environmental carcinogens are of major importance to the mission of the Cancer Program.

Proposed Course: The remaining projects in this contract have been or will be completed this Fiscal Year.

Date Contract Initiated: March 19, 1968

Current Annual Level: 0

NEBRASKA, UNIVERSITY OF (EPPLEY INSTITUTE FOR RESEARCH IN CANCER AND ALLIED DISEASES) (NO1-CP-05620) (Formerly NO1-CP 33278)

Title: Studies in Environmental Carcinogenesis Research and Bioassays (Skin Studies)

Contractor's Project Director: Dr. Ercole L. Cavalieri

Project Officer (NCI): Dr. David G. Longfellow

Objectives: To study the mechanisms of carcinogenesis of polycyclic aromatic hydrocarbons.

Major Findings:
I. One-electron Oxidation of Aromatic Hydrocarbons of Manganic Acetate -- In addition to the polycyclic aromatic hydrocarbons (PAH) previously studied, the hydrocarbons anthracene, pyrene, chrysene, 5-methylchrysene, perylene, anthanthrene and 6-methylanthanthrene were oxidized by one-electron oxidation with magnanic acetate in acetic acid at 40-55°C. Oxidation of anthracene for 24 h afforded cis-9,10-diacetoxy-9,10-dihydroanthracene (51%), trans-9,10-diacetoxy-9,10-dihydroanthracene (11%) and 9,10-anthraquinone (13%). Pyrene gave 1-acetoxypyrene (60%) and 1,6-diacetoxypyrene (16%) after 96 h. Chrysene did not react, while 5-methylchrysene yielded 6-acetoxy-5-methylchrysene (28%) after 96 h. Perylene in 10 min produced 1-acetoxyperylene (22%), 1,7-diacetoxyperylene (26%) and a

triacetoxyperylene (11%). Anthanthrene gave in 10 min 6-acetoxyanthanthrene (41%) and 6,12-diacetoxoyanthanthrene (12%), while 6-methylanthanthrene afforded only 12-acetoxy-6-methylanthanthrene. The attack by acetate ion on the PAH radical occurs, as expected, at positions of maximum charge density.

2. Synthesis of Radical Cations of Aromatic Hydrocarbons -- Benzo(a)pyrene (BP) and 6-methylbenzo(a)pyrene (BP-6-CH_3) radical cations were synthesized by oxidizing BP or BP-6-CH_3 in acetonitrile with iodine in the presence of silver perchlorate under argon atmosphere at room temperature. The reaction was complete in a few minutes. A black powder was obtained of $BP^{+}.ClO_4^{-}$ or BP-6-$CH_3^{+}.ClO_4^{-}$ mixed with silver iodide. The yields of BP and BP-6CH_3 radical cations by iodometric titration were 40% and 68%, respectively. $BP^{+}ClO_4^{-}$[3] and BP-6-$CH_3^{+}.ClO_4^{-}$ formed pyridimum derivatives by addition of pyridine. Reaction of the two radical cations perchlorates with DNA produced covalently bound DNA-adducts.

3. Horseradish Peroxidase/H_2O_2-Catalyzed Binding of PAH to DNA -- Further confirmation of one-electron oxidation as the mechanism of HRP/H_2O_2-catalyzed binding of PAH to DNA was obtained by examination of reaction mixtures. In this reaction mixture no metabolilte, including BP-6-CH_2OH, were detected by HPLC analysis of HRP/H_2O_2-catalyzed binding of BP-6-CH_3 to calf thymus DNA. This result is identical to the contractors previously published finding[2] that no metabolites are formed when BP is activated by HRP/H_2O_2.

The extent of HRP/H_2O_2-catalyzed binding of PAH has been found to correlate with the ionization potential (IP) of the PAH. A series of 14 PAH has now been studied with IP ranging from 8.19 to 6.68 eV. For the PAH phenanthrene, 5-methylchrysene, benzo(e)pyrene, dibenz(a,h)anthracene, benz(a)anthracene, pyrene, anthracene and 7-methylbenz(a)anthracene, with IP of 7.37 to 8.19, the level of binding varied between 1.4 and 8.8 μmol/mol DNA. In contrast, PAH with IP of 6.68 to 7.23, including benzo(a)pyrene, 7,12-dimethylbenz(a)anthracene, 3-methylcholanthrene, 6-methylbenzo(a)pyrene, anthanthrene and 6,12-dimethylanthanthrene, the extent of binding was 27 to 89 μmol/mol DNA. These results indicate the critical nature of the chemical properties of the PAH in determining the ease with which the binding reaction is catalyzed by HRP.

4. ATP-Mediated Binding of Hydroxymethyl PAH to DNA -- Mutagenesis by BP-6-CH_2OH and BA-7-CH_2OH in S. typhimurium in the presence of exogenous ATP was reported in last year's[2] "Highlights." The contractor has begun studies of the binding of hydroxymethyl PAH to DNA in these bacteria. Binding of (^{14}C)BP-6-CH_2OH and (^{14}C)BA-7-CH_2OH to DNA in S. typhimurium strain TA 98 grown 16 h with the PAH was dependent upon the concentration of PAH in the medium. At about 50 μM, binding of BP-6-CH_2OH was about 7.5 μmol/mol DNA while that of BA-7-CH_2OH was 1.5 μmol/mol DNA. Lower but significant levels of binding of both compounds were also obtained when bacteria were incubated with the PAH for 30 min. Bacterial replication was not essential for binding to occur. These studies are being continued to identify the DNA adducts formed and to relate binding to mutational events.

5. Mammary Carcinogenesis by Methylcholanthrene Derivatives -- Groups of 20 female Harlan Sprague-Dawley rats, 49-53 days old, were treated at two dose levels of 24 and 8 μmol of compound by "dusting" the fourth mammary gland on the right side after surgical incision in the mid-line. The rats were treated at two dose levels with methylcholanthrene (MC), MC-1-OH, MC-2-OH, MC-1-one, MC-2-one, MC-1-CH_3, MC-2-CH_3 or methylcholanthrylene (MCL). One group at the low dose level was treated with 7,12-dimethylbenz(a)anthracene (DMBA) as a positive control. After 30 experimental weeks

the experiment was terminated and the surviving rats were sacrificed. MC showed an observable tumor incidence in the fourth mammary gland region of 100% and 95% at the high and low dose, respectively. MC-2-OH had a 100% tumor incidence at both doses, while MC-1-OH displayed a 25% incidence only at the high dose. MC-2-CH_3 elicited a tumor incidence of 45 and 35 at the high and low dose, respectively, while MC-1-CH_3 was inactive. The high and low dose of MCL had a tumor incidence of 95% and 50%, respectively. DMBA at the low dose showed a 75% of tumor-bearing animals. All other compounds were inactive. These results, with the exception of the MC-1-OH, are consistent with MC activation by one-electron oxidation.

Significance To Biomedical Research and the Program of the Institute:
The complexity of polycyclic aromatic hydrocarbon (PAH) carcinogenesis can best be understood in terms of multiple mechanisms of activation. The data presently available indicate the important role of diol epoxides as ultimate carcinogenic metabolites. The main objective has been to determine the role of PAH radical cations and the PAH hydroxymethyl esters in PAH carcinogenesis. The discovery of the basic mechanisms of tumor initiation of these compounds is fundamental in terms of understanding the multistage induction process of cancer formation, as well as devising some strategy of possible prevention.

Proposed Course: The contract expired April 15, 1981.

Date Contract Initiated: March 19, 1968

Current Annual Level: 0

NEBRASKA, UNIVERSITY OF (EPPLEY INSTITUTE FOR RESEARCH IN CANCER AND ALLIED DISEASES) (NO1-CP-05621) (Formerly NO1-CP-33278)

Title: Studies on Developing Methods and on Causative Factors in Intestinal Carcinogenesis

Contractor's Project Director: Dr. Bela Toth

Project Officer (NCI): Dr. Carl E. Smith

Objectives: The specific purpose of the contract efforts is to develop appropriate models for colon carcinogenesis in mice in which carcinogenic exposure more nearly approximates possible human exposure; an additional objective is to investigate possible modifying factors for development of intestinal neoplasia.

Major Findings:
1. Benzo(a)pyrene (BP) was administered to Swiss mice in 1 and 10 weekly intracolonic instillations at 200 μg/g body weight. The single administration of BP induced a statistically significant incidence of malignant lymphomas and tumors of the forestomach, while its repeated instillation, evoked, in addition to these two tumor types, neoplasms in the esophagus, anus and skin.

BP failed to elicit intestinal tumors, which was the main objective of the present investigation. In light of the obtained results, the various experimental conditions under which studies were conducted on this most versatile and widely occurring carcinogen, to which a large segment of the human population is exposed, are described.

2. 4-Methylphenylhydrazine hydrochloride (4-MPH) was administered to randomly bred Swiss mice as 26 weekly subcutaneous injections of 140 µg/g body weight and N'-acetyl-4-(hydroxymethyl)-phenylhydrazine (AMPH) as 26 weekly subcutaneous injections of 500 µg/g body weight. As a solvent control, physiological saline (PS) was also given as 26 weekly subcutaneous injections of 0.01 ml/g body weight. The 4-MPH treatment induced a significant incidence (24%) of fibrosarcomas in males. In the 4-MPH-treated females and some AMPH-treated male mice, a few soft tissue tumors were observed; however, their appearance could not be related to treatment.

4-MPH is formed under special experimental conditions from 4-hydroxy-methylphenylhydrazine, which is an in vitro breakdown product of agaritine, an ingredient of the cultivated mushroom Agaricus bisporus. The implications of the findings are described.

3. Data are summarized on 13 hydrazines and related -N-N-bond-containing chemicals that induce tumors in the large intestine, mainly the colon, of laboratory animals. These compounds belong to the hydrazine, azoxy and N-nitroso classes and include: azoxymethane (AM), 1,1-dimethylhydrazine (1,1-DMH), 1,2-dimethylhydrazine dihydrochloride (1,2-DMH), formic acid 2-[4-(5-nitro-2-furyl)-2-thiazolyl hydrazide (FAH), methylhydrazine (MH), ONN-methylazoxybutane (MAB), methylazoxymethanol (MAM), methylazoxymethanol glycoside (MAMG), 1-methyl-2-butylhydrazine dihydrochloride (MBH), N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), N-nitrosobis(2-hydroxypropyl)amine (BHP), N-nitrosobis(2-oxopropyl)amine (BOP) and trimethylhydrazine hydrochloride (TMH). Of these, the following four or their precursors occur in nature: 1,1-DMH, MH, MAM, and MAMG. 1,1-DMH is an ingredient of tobacco and tobacco smoke. MAMG and its degradation product MAM are components of cycad nuts, especially of Cycas circindalis L., which are used as food in the tropics. MH was detected in one of the edible canned false morel mushrooms Gyromitra esculenta. Two chemicals of this series, 1,1-DMH and MH are manufactured synthetically and used in rocket fuels, as well as other ways. Therefore, to some extent, in certain parts of the world the human population is exposed to these four chemicals. The remaining nine compounds, AM, 1,2-DMH, FAH, MAB, MBH, MNNG, BHP, BOP and TMH are made synthetically in limited quantities only for laboratory use. All 13 chemicals induce tumors in the large intestine, mainly in the colon, and also in the rectum and cecum of mice, hamsters and rats. In addition, they also elicit tumor development in the following tissues: lungs, blood vessels, breasts, kidneys, external auditory canal, bile ducts, small intestine, liver, stomach, esophagus, pharynx, thyroid, bladder, urethra, bulbus olfactorius, Kupffer cells, etc. The environmental significance of some of these intestinal cancer-inducing agents is evident.

Significance to Biomedical Research and the Program of the Institute:
Carcinogenesis systems more closely modeling the human experience are critically needed for basic studies of cancer development and approaches to anti-carcinogenesis.

Proposed Course: It is a phase-out contract, and the ongoing studies should be evaluated by the end of the contract year.

Date Contract Initiated: March 19, 1968

Current Annual Level: 0

NEBRASKA, UNIVERSITY OF (EPPLEY INSTITUTE FOR RESEARCH IN CANCER AND ALLIED DISEASES) (NO1-CP-05622) (Formerly NO1-CP-33278)

Title: Action of Tryptophan on the Bladder

Contractor's Project Director: Dr. Terence Lawson

Project Officer (NCI): Dr. Carl E. Smith

Objectives: Determination of the effects of subchronic administration of N-hydroxy derivatives of tryptophan upon bladder epithelial cell proliferation. Determination of preliminary pharmacokinetic data on blood and urine levels of these compounds.

Major Findings: This project was based on two ideas:

1. Aromatic amines that are carcinogenic in the urinary bladder act via the N-hydroxy derivative.

2. Chemicals that are carcinogenic to the urinary bladder produce bladder epithelial hyperplasia and an increase in bladder epithelial DNA synthesis on sub-chronic administration.

Linking these two ideas together, it was proposed that the carcinogenicity of tryptophan to the urinary bladder epithelium in rats was mediated through an N-hydroxy derivative which on prolonged administration (10 daily intraperitoneal injections) would produce an increase in bladder epithelial DNA synthesis. Of the six compounds originally proposed, only N-hydroxy anthracilic acid (NHA) was synthesized; and it was found to be very labile. The LD_{50} of NHA on i.p. administration was found to be 500 mg/kg in both male and female MRC-Wistar rats. Single large doses of NHA (50 or 200 mg/kg) produced an increase in bladder epithelial DNA synthesis but only in male rats. When given orally, NHA was ineffective in increasing bladder epithelial DNA synthesis.

Sub-chronic administration of low doses (10 daily injections over a twelve-day period) of NHA (up to 5% LD_{50}) failed to increase bladder epithelial DNA snthesis. DNA synthesis was determined by measuring the incorporation of ^{3}H-thymidine in bladder epithelial DNA. Both autoradiography and chemical extraction of the epithelial DNA and measurement of the specific activity of the isolated DNA were used.

Significance to Biomedical Research and the Program of the Institute:
These experiments represented a new approach to the involvement of tryptophan in bladder carcinogenesis. The extreme lability of the compounds precluded a study of all but one compound (NHA). It must be concluded it is unlikely that NHA would be a bladder carcinogen. Furthermore, its failure to produce comparable effects to those obtained when given by i.p. injection when given orally, indicate that it is unlikely to be involved in the role of ingested tryptophan in human bladder cancer.

Proposed Course: The contract ended November 15, 1980.

Date Contract Initiated: March 19, 1968

Current Annual Level: 0

NEBRASKA, UNIVERSITY OF (EPPLEY INSTITUTE FOR RESEARCH IN CANCER AND ALLIED DISEASES) (NOI-CP-05623) (Formerly NOI-CP-33278)

Title: A Study of Prenatal Carcinogenicity of Anti-Fertility Drugs: Enovid-E, Loestrin, Micronor, Oracon, and Estradiol-B

Contractor's Project Director: Dr. David Clayson

Project Officer (NCI): Dr. Carl E. Smith

Objectives: To evaluate, for carcinogenic response, selected contraceptive drugs administered prenatally and postnatally in the hamster.

Major Findings: The anti-fertility drugs selected for study were Enovid-E, Oracon, Loestrin, and Norethindrone, as well as the natural estrogen, estradiol-17β. All the test animals are dead, all tissues cut, processed, and slides prepared for histopathological evaluation. So far, results are available only for Enovid-E. This contraceptive drug fed daily in the diet for life results in significantly higher average tumor incidences (tumors of any type) in both males and females than in control groups. In females, the increase in tumor incidence is primarily due to a significant frequency of uterine polyps. Most of these benign tumors were associated with glandular cystic hyperplasia and multiple polypoid projections. Hyperplastic and metaplastic lesions, considered potentially malignant, were frequently encountered, occasionally with atypia. In general, this contraceptive drug affected primarily target tissues or organs that are under the endocrine control of steroid hormones.

Significance to Biomedical Research and the Program of the Institute: Contraceptive drugs of this type have had widespread human use. Several anti-fertility drugs are known to be tumorigenic in mice, and estrogens are known to have transplacental carcinogenic activity in humans. The hamster is sensitive to estrogens and responds readily by neoplastic development. For these reasons, and since the chronological order of different developmental stages during prenatal ontogenetic development is well-known in the hamster, continued study of these compounds in this species for possible developmental abnormalities or neoplastic effects is of the highest importance.

Proposed Course: The former principal investigator (Dr.Rustia) has left the Eppley Institute, and the contract there has terminated.

Date Contract Initiated: March 19, 1968

Current Annual Level: 0

NEBRASKA, UNIVERSITY OF (EPPLEY INSTITUTE FOR RESEARCH IN CANCER AND ALLIED DISEASES) (NOI-CP-05624) (Formerly NOI-CP-33278)

Title: The Effect of Oral Contraceptive Steroid Treatment on Carcinogen Metabolism in Rats and Hamsters

Contractor's Project Directors: Dr. Ralph Gingell
Dr. Terence Lawson

Project Officer (NCI): Dr. Carl E. Smith

Objectives: To account for the observation that female hamsters maintained on Enovid or treated transplacentally with diethylstilbestrol (DES) develop a much higher incidence of mammary tumors when also exposed to 7,12-dimethyl-benz(a)anthracene (DMBA) than hamsters exposed to DMBA alone.

Major Findings: No overt differences were observed in the in vivo metabolism and distribution of DMBA in hamsters as a result of hormone pretreatment, although Enovid did induce cholestasis in rats. Also, in vivo hepatic and mammary aryl hydrocarbon hydroxylase activities were not affected by these hormone pretreatments, although other known modifiers of metabolism were effective. Metabollic activity in the tissues of rat, which is very sensitive to DMBA-induced mammary tumors, is much less than that observed in the same tissues of the hamster. These results suggest that any effect of hormone pretreatment may be on the qualitative nature of DMBA metabolites. Mammary DNA synthesis, as measured by thymidine incorporation, varied with the day of the estrous cycle in young animals; and this variation was abolished by acute Enovid pretreatment. Neither the overall extent nor persistence of binding of DMBA to mammary DNA was different in the control, Enovid, or DES pretreated animals. Thus, the difference in mammary tumor induction by DMBA in these different hormonal states may be associated with the qualitative nature of the bound adducts. However, the metabolism of DMBA was not different in Enovid treated hamsters. Compared with control hamsters, microsomal preparations from Enovid treated hamsters did not metabolize DMBA any differently than those from control hamsters. These results support the idea that Enovid must be affecting another phase of initiation, or more likely, the development of mammary tumors.

Significance to Biomedical Research and the Program of the Institute: Experimental studies of mechanisms of mammary tumor induction and factors which may modify this mechanism, might yield information important to the understanding of the cause of the human diseases, and thus, to preventative measures.

Proposed Course: The contract ended November 15, 1980.

Date Contract Initiated: March 19, 1968

Current Annual Level: 0

NEBRASKA, UNIVERSITY OF (EPPLEY INSTITUTE FOR RESEARCH IN CANCER AND ALLIED DISEASES) (NO1-CP-05625) (Formerly NO1-CP-33278)

Title: Establishment of Mouse Urinary Bladder Epithelial Cells in Culture

Contractor's Project Director: Dr. Linda E. Malick

Project Officer (NCI): Dr. David G. Longfellow

Objectives: To develop a urinary bladder epithelial cell-mediated mutagenesis system using Chinese hamster V79 cells as the mutable indicators. This system would provide a means of studying the ability of bladder epithelial cells to metabolize potential and/or known carcinogens to their activated forms, thus providing a system for examination of their mechanisms of action.

Major Findings: Potential and known chemical carcinogens tested for metabolic activation by urinary bladder epithelial cells in the presence of V79 cells were also accompanied by corresponding tests on V79 cells without bladder cell activation. 7,12-Dimethylbenz(a)anthracene, benzo(a)pyrene, dimethylnitrosamine, dibutylnitrosamine and B-naphthylamine produced a significant number of mutants with bladder cells present and few or no mutants when V79 cells alone were treated in the same tests. This demonstrates that the urinary bladder epithelial cells have the capacity to metabolize substances from different chemical groups to their active (mutagenic) forms. Comparative tests with different species indicate that there are species differences in the metabolizing ability of urinary bladder epithelial cells from mouse, rat and cow. The metabolic capabilities of kidney cells in culture to activate the same chemicals to mutagenic metabolites were also studied. In the presence of bladder cells, cyclophosphamide induced a greater number of mutants using 6-thioguanine as the genetic marker than with ouabain. However, a significant number of mutants were also induced without bladder cell activation, raising the question of whether the presence of the bladder cells is necessary in this situation and/or whether different metabolic pathways are utilized in the presence or absence of bladder cells. In addition, results indicate that cell-cell interactions play a role in cell-mediated mutagenesis systems. The ability of some glycoproteins to enhance the mutagenic response was investigated.

Significance to Biomedical Research and the Program of the Institute:
Use of urinary bladder epithelilum and other cell types in tissue culture provide a means of determining the effect of chemical carcinogens, hormones, drugs and other substances on specific organ and cell types with the aim of providing a better understanding of the cellular mechanisms and biological properties involved in carcinogen-induced cell alterations. Methods which enhance cell-mediated mutagenesis levels increase the sensitivity of such assay systems providing a better means of detecting substances potentially hazardous to humans.

Proposed Course: The contract was completed November 15, 1980.

Date Contract Initiated: March 19, 1968

Current Annual Level: 0

NEBRASKA, UNIVERSITY OF (EPPLEY INSTITUTE FOR RESEARCH IN CANCER AND ALLIED DISEASES) (NO1-CP-05627) (Formerly NO1-CP-33278)

Title: Activation and Transport of N-Nitrosamines and Their Metabolites

Contractor's Project Director: Dr. Barry Gold

Project Officer (NCI): Dr. David G. Longfellow

Objectives: To obtain additional evidence for the involvement of α-hydroxylation in the carcinogenic activation of secondary nitrosamines, and to determine the site of this enzymic step.

Major Findings:
1. It has been demonstrated that α-hydroxynitrosamines, the proposed proximate carcinogenic form in nitrosamine carcinogenesis, are sufficiently stable to be

formed outside the nuclear envelope and migrate into the nucleus prior to decomposing. Evidence has also been obtained which indicates that formation of the α-hydroxynitrosamine intermediate at the nuclear envelope by nuclear membrane-bound enzymes is much more efficient in forming alkylated DNA adducts than by cytolasmic microsomal enzymes.

2. It has been demonstrated that the base-catalyzed decomposition of N-nitrosocarbamates at near physiological pH is initiated by hydroxide attack at the nitroso nitrogen rather than at the carbonyl carbon.

3. The chronic tests of acetoxymethyl-t-butylnitrosamine and methyl-t-butylnitrosamine in Syrian hamsters have been completed and, as expected, both compounds were inactive. These results are taken to indicate that t-butyl carbenium ion cannot interact with the critical cellular site(s) to initiate either carcinogenesis or mutagenesis.

4. The chronic testing of acetoxymethylphenylnitrosamine and benzenediazonium tetrafluoroborate in the Syrian golden hamster has been completed. The former compound is a weak local acting carcinogen, while the latter did not afford a significant number of tumors either by subcutaneous or intragastric administration.

Significance to Biomedical Research and the Program of the Institute:
The results obtained provide information concerning the reasons why and how nitrosamines initiate carcinogenesis in laboratory animals. The bioassay results indicate that arenediazonium ions, which are present in the environment and of commercial utility, should be considered as direct acting carcinogens.

Proposed Course: The contract expired January 15, 1981.

Date Contract Initiated: March 19, 1968

Current Annual Level: 0

NEBRASKA, UNIVERSITY OF (EPPLEY INSTITUTE FOR RESEARCH IN CANCER AND ALLIED DISEASES (NO1-CP-05628) (Formerly NO1-CP-33278)

Title: The Possible Influence of Diet in Carcinogenesis

Contractor's Project Director: Dr. Diane D. Birt

Project Officer (NCI): Dr. Carl E. Smith

Objectives:
1. The requirement of hamsters for several well-utilized dietary proteins will be established. Each protein will be fed at four levels and measurements made of nitrogen utilization, blood, proteins, and hepatic drug metabolism.

2. These studies will serve as a basis for selecting three levels of a well-utilized protein: one level near the requirement for maximal growth and one level each above and below this requirement.

3. These diets will be fed, from weaning, to three groups of male and female hamsters (F_0 generation), and additional groups will be fed the commercial diet.

4. The parent generation will be bred with mates fed the same diet. The offspring (F_1 generation) will be fed the diet of their parents for life.

5. Complete histopathological evaluation of animals fed each of the diets will be conducted.

6. The nutritional status will be measured of a random sample of animals fed each of the diets during each generation. Kill animals at 0, 6, 18, 30 and 60 weeks after weaning and determine carcass nitrogen, some blood proteins, and hepatic microsomal cytochrome P_{450} and the activities of some microsome drug-metabolizing enzymes.

7. Malignant and non-malignant disease to diets consumed will be related and age adjustment used to correct for and interpret differences in longevity.

8. Changes will be related in disease and longevity between animals fed different diets with the effects of these diets on the nutritional health and toxicological capabilities of the hamsters.

Major Findings:

1. The parent generation (F_0) is over 110 weeks old and less than 2% are still surviving. The youngest animals in the F_1 generation are 65 weeks old and less than 20% of this generation remains alive. Statistical comparison of the F_0 generation has demonstrated the longest survival in hamsters fed the medium protein level (20%), and the poorest in those fed the commercial ration.

2. Histopathological observations have been made on approximately 25% of the F_0 generation. Preliminary observations indicate a possible difference in disease patterns between animals receiving the different diets.

3. Nutritional and toxicological parameters have been evaluated in animals killed at 0, 6, 18, 30 and 60 weeks from the parent generation, and from all of these times, except at 60 weeks for the F_1 generation. The 60-week F_1 killings were completed and samples are being analyzed. A general trend is apparent in both generations in terms of body weight,, serum albumin, microsomal protein and the activities of hepatic microsomal aniline hydroxylase and benzo(a)pyrene hydroxylase. In each case, increases in these values were observed with increases in dietary protein for young animals, but in older animals the highest values were observed in those fed the medium level of protein.

4. Differences between the two generations were most prominent for the hepatic microsomal components measured. Cytochrome P_{450} and aniline hydroxylase activity were higher during several intervals in the F_1 generation; however, benzo(a)pyrene hydroxylase activity was lower during several intervals in the F_1 generation.

Significance to Biomedical Research and the Program of the Institute:

Nutritional factors have been implicated in human and experimental cancer. Dietary protein has been shown to play a role in induced and spontaneous carcinogenesis. However, results from different studies do not agree. Studies of spontaneous tumors are particularly relevant, since human and animal tumors are probably caused by some of the same agents. On the other hand, few studies have investigated the effects of diet on spontaneous tumors; and none have used hamsters. Furthermore, previous studies have begun with animals at weaning, while two-generation studies on the effects of diet on spontaneous tumors have not been reported.

This project investigates the influence of dietary protein on spontaneous tumors in two generations of Syrian hamsters. Additional hamsters fed each diet during the two generations were killed and measurements of nutritional status and in vitro drug metabolism were conducted. These determinations indicated responses of the hamsters to the level of protein consumed, which may be related to the tumor and disease patterns observed in the animals. An understanding of health and nutritional status of animals on chronic carcinogenesis studies will assist in relating the results to comparable studies with other animals, and in eventually extending the information gained to humans.

Proposed Course:

1. Remaining survivors will be allowed to die, and evaluation of the effects of diet on longevity will be completed.

2. Complete histopathological observations on malignant and non-malignant diseases in both generations.

3. Relate the differences in disease patterns between animals fed the various diets to the effects of the diets on longevity (this will require age and adjustment of the pathological data).

4. Use the information gathered on the effects of the diets on nutritional status and drug metabolism throughout aging to assist in the interpretation of changes in disease patterns and longevity.

Date Contract Initiated: March 19, 1968

Current Annual Level: $64,086

NORTH CAROLINA, UNIVERSITY OF (NO1-CP-75956)

Title: Studies of Carcinogenesis in Human Tissue

Contractor's Project Directors: Dr. David G. Kaufman
Dr. Leslie A. Walton

Project Officer (NCI): Dr. Lea I. Sekely

Objectives: The overall objective of this project is to determine whether specific insights into the causation of human endometrial carcinoma can be derived by direct study of the effects of chemical carcinogens on human endometrial tissue. Specific objectives for the current year of this project were to further characterize the biologic properties of this tissue in vitro and to evaluate the effects of chemical carcinogens on human endometrial tissue in culture.

Major Findings: Appropriate methods have been utilized for obtaining human endometrial tissue from surgical hysterectomy specimens. Both cell and organ cultures have been established with high frequencies and with persistance of selected organ and cell cultures in vitro for periods exceeding a year. Organ culture studies have focused on variations in carcinogen metabolism and binding as

functions of physiologic cycles of estrogens and progestins. Studies of metabolism and binding of benzo(a)pyrene (BP) in fresh endometrial tissue have shown that the state of estrogenization of this tissue affects both parameters. Tissue from postmenopausal women exhibits reduced proportions of BP sulfate conjugates and BP tetrol metabolites and also have lower levels of binding of BP to DNA in comparison to average values for premenopausal women. Conversely, for premeropausal women, the proportions of BP sulfate conjugates and tetrols were highest and BP binding to DNA peaked in the middle third of the menstrual cycle. These observations show that for this human tissue, some of the variability in BP metabolism and part of the range of observed values of binding of BP to DNA in vitro may be explained by the physiologic status (i.e., hormonal) of the tissue.

A number of studies have been undertaken to improve and further characterize cell and organ cultures. Cell cultures have been subjected to more intense electron microscopic study. Mature glandular epithelial cells and myofibroblasts had characteristic ultrastructural features. Type 1 cells, presumed to be immature epithelial cells, showed complex interdigitations with numerous gap junctions and had microvilli on their free surfaces. Two other morphologically distinct cell types also were insufficiently well differentiated to determine their origin. Type 1 cells were shown to have optimal growth in 20% fetal bovine serum but could be grown for at least a month in hormone-supplemented serum-free medium and were stimulated by epidermal growth factor. Efforts have been made to extend the growth and survival of mature epithelial cells in culture. Endometrial glands have been isolated enzymatically and used both as a source for cell cultures of mature epithelial cells and as an alternative to organ cultures. Studies with organ cultures have attempted to improve survival of stromal elements by culturing on a rocker platform and by increasing the oxygen content of the gas phase of the cultures.

The major focus of this project now concerns attempts to malignantly transform human endometrial tissue by chemical carcinogen treatments in culture. Type 1 cell cultures have been subjected to repetitive brief exposures to MNNG and several growth properties, and cellular characteristics have been evaluated following each treatment. Studies with a number of cultures and subcultures of Type 1 cells from several patients have shown similar sequential changes occurring with increasing numbers of treatments. Although initial treatments are toxic and reduce the numbers of viable cells and their growth rates, cultures emerge with increased plating efficiency, growth rate, and saturation density. Treated cells develop increased levels of gamma glutamyl transpeptidase activity and are able to grow as colonies in soft agar. The morphologic appearance of the cells undergoes a progressive change with substantial increase in the nucleus/cytoplasm ratio. These cells have been injected into nude mice but, to the present, no tumors have developed in the recipients. Efforts continue to attempt to obtain tumorous growth by further treating the cells and by injecting into immunologically privileged sites or using further immunologically compromised nude mice. Other studies of carcinogen treatment of endometrial tissue include treatment of cells in primary culture or of isolated glands or organ cultures followed by enzymatic dissociation and cell culture. Initial studies with TPA have shown this promoter to stimulate growth of endometrial Type 1 cell cultures.

Significance to Biomedical Research and the Program of the Institute:
The overall objective of this program is to malignantly transform human endometrial tissue by treatment with chemical carcinogens in vitro. These studies may contribute to our understanding of the causation of human endometrial cancer and

help explain interindividual variation in susceptibility to this disease. The accomplishments to date in the performance of this contract represent tangible steps in efforts to achieve these objectives.

Proposed Course: During the remainder of this year of the contract, several lines of investigation will be given emphasis. Efforts will continue in attempts to morphologically and biochemically characterize Type 1 cells. Further attempts will be made to establish clonal cultures of these cells and to obtain prolonged survival and growth of mature epithelial cells. Three dimensional structural matrices maintained as xenografts in vivo will be evaluated as a test system for expression of differentiated properties of previously cultured cells. Studies will continue to characterize the responses of Type 1 cell cultures to carcinogen treatments and to TPA and the effects of carcinogen treatment of primary cultures, isolated glands and organ cultures will be further explored. Studies with nude mice will continue the evaluation of growth of carcinogen-treated cells injected into these animals. Concurrently, efforts will be made to determine the fate of injected cells and to evaluate the immune response in these mice.

Date Contract Initiated: September 30, 1977

Current Annual Level: O

TEMPLE UNIVERSITY (NO1-CP-85603)

Title: Hairless Mice for UV Carcinogenesis Studies

Contractor's Project Director: Dr. Paul Donald Forbes

Project Officers (NCI): Dr. Morris Kelsey
Dr. David G. Longfellow

Objectives: The overall objectives of this project include:

1. Deriving the comparative UV photocarcinogenic sensitivity of selected stocks and strains of non-haired mice.

2. Characterizing several immunologic traits of the selected animals.

3. Evaluating breeding efficiency of stocks or strains which might be designated as preferred test organisms for photocarcinogenesis studies.

4. Completing special studies on four of the strains.

Major Findings: The primary purpose of this contract was to examine several strains of hairless mice in order to select and develop appropriate animals for work in the area of photobiology and photocarcinogenesis. By the time the project was well under way, the inbred strain designated HRA/Skh had emerged as a particularly attractive animal model. These mice are robust and prolific, overcoming many of the economic difficulties and handling disadvantages of hairless mice. This strain of animals is currently being used in the National Toxicology Program's bioassay of 8-methoxypsoralen and related psoralen derivatives. Although the HRA/Skh strain is no more acutely sensitive to ultraviolet radiation than a number of other stocks and strains of hairless mice, this strain is particularly prone to early skin tumor

formation following repeated exposure to ultraviolet radiation. Each of ten stocks and strains of non-haired mice is being subjected to a dose-response study, involving five different daily doses of simulated sunlight. The ten representative stocks and strains each appear to have a characteristic sensitivity, and the sensitivities form a continuum from the HRA/Skh at one extreme to the HRS/J at the other extreme. For example, HRS/J animals require about twice the dose that is needed to produce a given tumor response in HRA/Skh mice. A number of immunologic responses have been evaluated in each of these stocks and strains, and at least in the unirradiated mice, the derived values are within normal limits. The tests included the ability to respond to various mitogens as well as to T-cell dependent and T-cell independent antigens. The numbers of T lymphocytes and B lymphocytes in the spleen tymus and lymph nodes were found to be comparable among the strains. The antibody forming ability as measured by plaque-forming cells in the spleen was demonstrable in all strains. These responses are now being evaluated in UVR-irradiated mice. Animals are also being tested for their ability to mount a delayed contact-sensitivity response, and the contractor is looking for other sources of their dissimilar UVR cancer susceptibility, such as subtle differences in skin structure, in epidermal cell kinetics, and in their vascular responses.

Significance to Biomedical Research and the Program of the Institute: Heritable factors influence susceptibility to carcinogens, as clearly evidenced in animal studies. This study identifies several sources of material for isolating and evaluating such components as immunologic and cellular repair.

Proposed Course: Several projects will be completed during this contract year. The UVR dose-response data on the tested strains will be analyzed and compared. Pathology specimens will be examined for differences in tumor type. Special studies on vascular responses and on epidermal cell kinetics in selected strains will be completed. A battery of immunologic test on UV-irradiated animals will be finished. This work has uncovered some promising leads which will be incorporated into recommendations for future support.

Date Contract Initiated: September 1, 1978

Current Annual Level: 0

CHEMICAL AND PHYSICAL CARCINOGENESIS BRANCH

GRANTS ACTIVE DURING FY 81

CARCINOGENESIS MECHANISMS

Investigator/Institution/Grant Number	Title
ALEXANDER, Chester, Jr. University of Alabama in University 5 RO1 CA 20245-02	Free Radical Reactions in DNA Purine Analogs
ALWORTH, William L. Tulane University of Louisiana 5 RO1 CA 23014-03	Modification of Mammalian Epoxide Hydrase Activity
ALWORTH, William L. Tulane University of Louisiana 5 RO1 CA 23014-02	Modification of Mammalian Epoxide Hydrase Activity
ANDERSON, John N. Purdue University West Lafayette 1 RO1 CA 25799-01	Conformation of Alpha-Fetoprotein Gene in Chromatin
ANDERSON, Lucy M. Sloan-Kettering Institute for Cancer Research 2 RO1 CA 22509-04	Alcohol and Cancer
ANDERSON Lucy M. Sloan Kettering Institute for Cancer Research 5 RO1 CA 22509-03	Chemical Factors in Fetal Oncogenesis
ARCHER, Michael C. Ontario Cancer Institute 5 RO1 CA 26651-02	Mechanism of Nitrosamine Alkylation of DNA and RNA
ASTLE, Lynn University of Utah 1 RO1 CA 28006-01	Gastrointestinal Carcinogenicity of Malondialdehyde
BAIRD, William M. Purdue University West Lafayette 2 RO1 CA 28825-02	Modifiers of Chemical Carcinogenesis in Cell Culture
BECKER, Frederick F. University of Texas System Cancer Center 2 RO1 CA 20659-05	Analysis of Cellular Events in Chemical Carcinogenesis
BOYLAN, Elizabeth S. Queens College 5 RO1 CA 18458-05	Fetal Exposure to Hormones and Mammary Carcinogenesis

BRADA, Zbynek
Papanicolaou Cancer Research Institute
5 RO1 CA 23516-03

Ethionine Metabolism in Hyperplastic Nodules in Liver

BRAND, K. Gerhard
University of Minnesota of Minneapolis-St. Paul
5 RO1 CA 10712-13

Sequential Cell Studies in Foreign Body Tumorigenesis

BRESNICK, Edward
University of Vermont
5 RO1 CA 20711-05

Polycyclic Hydrocarbon Metabolism and Carcinogenesis

BUHLER, Donald R.
Oregon State University
5 RO1 CA 22524-04

Metabolism and Toxicity of Pyrrolizidine Alkaloids

COATES, Robert M.
University of Illinois Urbana-Champaign
5 RO1 CA 20436-05

Hydroxylamine Rearrangements and Carcinogenesis

COHEN, Arthur M.
University of Southern California
5 RO1 CA 24518-03

Inhibition of Carcinogenesis by Selenium

COLBY, Howard D.
West Virginia University
5 RO1 CA 22152-03

Adrenal Carcinogen Metabolism

CORBETT, Michael D.
University of Miami
2 RO1 CA 21992-04

Hydroxamic Acid Production by Marine Organisms

DAUB, Guido H.
University of New Mexico
5 RO1 CA 16871-03

^{13}C-Enriched Chemical Carcinogens

DIAMOND, Leila
Wistar Institute of Anatomy and Biology
5 RO1 CA 08936-12

The Effect of Chemical Carcinogens on Cells In Vitro

ERNSTER, Lars
University of Stockholm
5 RO1 CA 26261-02

Metabolism of Polycyclic Hydrocarbons and Cancer

FIALA, Emerich S.
American Health Foundation
5 RO1 CA 26395-02

Single Ring Arylamine Carcinogens: Mechanism of Action

FLETCHER, T. Lloyd
Fred Hutchinson Cancer Research Center
5 RO1 CA 19279-03

Approaches to Chemical Carcinogenesis Mechanisms

FLOYD, Robert A.
Oklahoma Medical Research Foundation
5 R01 CA 18591-06

Carcinogen Free Radicals in Arylamine Metabolism

FLOYD, Robert A.
Oklahoma Medical Research Foundation
5 R01 CA 21542-03

Free Radicals in Nitroso-Unsaturated Lipid Reactions

FRANK, Arthur L.
Mount Sinai School of Medicine
5 R23 CA 24271-03

Mineral Fiber Size and Carcinogenicity In Vitro

GIBSON, David T.
University of Texas Austin
5 R01 CA 19078-06

Microbial Degradation of Carcinogenic Hydrocarbons

GLUSKER, Jenny P.
Institute for Cancer Research
2 R01 CA 10925-32

Application of Crystallographic Techniques

GOLD, Avram
University of North Carolina Chapel Hill
1 R01 CA 28622-01

Activation of Polycyclic Environmental Mutagens

GOLD, Barry I.
University of Nebraska Medical Center
5 R01 CA 24554-03

Epoxidation in Chloro-Olefin Carcinogenesis

GOLDMAN, Peter
Beth Israel Hospital
5 R01 CA 15260-08

Carcinogen Metabolism by Host Intestinal Bacteria

GOULD, Michael N.
University of Wisconsin Madison
1 R01 CA 28954-01

Carcinogen Activation by Cultured Mammary Cells

GREENBERGER, Joel S.
Sidney Farber Cancer Institute
5 R01 CA 25412-03

Stem Cell Age and X-Ray/ Chemotherapy Leukemogenesis

GRIBBLE, Gordon W.
Dartmouth College
5 R01 CA 24422-03

Fluorinated Benzanthracene Synthesis and Screening

GURTOO, Hira L.
Roswell Park Memorial Institute
5 R01 CA 25362-02

Genetics of Aflatoxin Metabolism--Role in Carcinogenesis

GUTTENPLAN, Joseph B.
New York University
5 R01 CA 19023-05

Mechanism of Mutagenesis and Carcinogenesis

HANRATTY, William P. University of California Irvine 5 RO1 CA 24488-02	The Controlled Initiation of Neoplasms in Drosophila
HARPER, Curtis University of North Carolina Chapel Hill 5 RO1 CA 21705-03	Environmental Organohalogens Lung Toxicity
HARVEY, Ronald G. University of Chicago 5 RO1 CA 11968-09	Chemistry of Carcinogenic Hydrocarbons
HECHT, Stephen S. American Health Foundation 5 RO1 CA 23901-03	Environmental Nitrosamines--Metabolism & Carcinogenesis
IANNACCONE, Philip M. Northwestern University 1 RO1 CA 29675-01	Effects of Exposure to Carcinogens on Blastocysts
IRVING, Charles C. University of Tennessee Center for Health Sciences 5 RO1 CA 26165-02	Conjugation Reactions in Arylamine Carcinogenesis
JIRTLE, Randy L. Duke University 5 RO1 CA 25951-02	Survival and Carcinogenesis in Transplanted Hepatocytes
JUNGALWALA, Firoze B. Eunice Kennedy Shriver Center for Mental Retardation 5 RO1 CA 16853-05	Biochemical Aspects of Experimental Brain Tumors
KAUFFMAN, Frederick C. University of Maryland at Baltimore 2 RO1 CA 20807-04	Pharmacology of Carcinogen Activation in Intact Cells
KOESTNER, Adalbert Ohio State University 2 RO1 CA 11224-11A1	Neuro Oncogenesis by Resorptive Carcinogens
LEHR, Roland E. University of Oklahoma Norman 5 RO1 CA 22985-05	Diol Epoxide/Other Derivatives of PAH and aza-PAH:SAR's
LEVINE, Walter G. Yeshiva University 5 RO1 CA 14231-09	Role of Metabolism in the Biliary Excretion of Drugs
LILL, Patsy H. University of South Carolina at Columbia 5 RO1 CA 25361-03	Carcinogen Induced Facilitation of Tumor Growth

LIPKIN, Martin Sloan-Kettering Institute for Cancer Research 1 R01 CA 28805-01	Nitrate Metabolism in Gastrointestinal Cancer
LOEPPKY, Richard N. University of Missouri Columbia 2 R01 CA 22289-04	Nitrosamine Fragmentation and Nitrosamine Carcinogenesis
LOEPPKY, Richard N. University of Missouri Columbia 5 R01 CA 26914-02	Carcinogenesis: Nitrosamine Formation and Inhibition
LOTLIKAR, Prabhakar D. Temple University 5 R01 CA 10604-14	Mechanism of Chemical Carcinogenesis
MAGEE, Peter N. Temple University 5 R01 CA 23451-03	Formation and Metabolism of N-Nitroso Compounds
MALEJKA-GIGANTI, Danuta University of Minnesota of Minneapolis-St. Paul 5 R01 CA 28000-02	Mammary Carcinogenesis by Arylhydroxamic Acids
MANDEL, Richard Boston University 5 R01 CA 27324-02	Additive and Synergistic Effects of Mutagens
MARNETT, Lawrence J. Wayne State University 5 R01 CA 22206-03	Malonaldehyde Studies
MAYS Charles W. University of Utah 1 R01 CA 28314-01	Reducing Cancer Risk by Radionuclide Chelation
MC MURTREY, Kenneth D. University of Southern Mississippi 1 R01 CA 29903-01	Toxicology of Polynuclear Heterocyclic Carcinogens
MILLER, Richard K. University of Rochester 5 R01 CA 22335-04	Transplacental Carcinogenesis
MIRVISH, Sidney S. University of Nebraska Medical Center 5 R01 CA 24776-02	Mechanism of Liver Carcinogenesis by Two Nitrosoureas
MOOLTEN, Frederick L. Boston University 5 R01 CA 23534-03	Protective Immunity to Chemical Carcinogens

MORREAL, Charles E.
Roswell Park Memorial Institute
5 R01 CA 22001-03

Biological and Chemical Studies of New DMBA Compounds

MORRISON, Harry A.
Purdue University West Lafayette
2 R01 CA 18267-04

Cutaneous Photobiology and Drug Phototoxicity

NEWMAN, Melvin S.
Ohio State University
5 R01 CA 07394-16

Synthesis of Substituted Polycyclic Hydrocarbons

NORBACK, Diane H.
University of Wisconsin Madison
5 R01 CA 22140-03

Carcinogenic Action of Polychlorinated Biphenyls

O'FLAHERTY, Ellen J.
University of Cincinnati
1 R01 CA 29917-01

Quantitative Considerations in Urethan Carcinogenesis

OLIVE, Peggy L.
Johns Hopkins University
5 R01 CA 24519-03

Activation and Reactivity of Nitroheterocycles

PAIGEN, Beverly J.
Roswell Park Memorial Institute
5 R01 CA 24270-03

Genetic Susceptibility to Lung Cancer

PAQUETTE, Leo A.
Ohio State University
2 R01 CA 12115-11

Unsaturated Polyolefins and Hydrocarbon Carcinogenesis

PARTHASARATHY, Rengachary
Roswell Park Memorial Institute
2 R01 CA 23704-04A1

Stereochemistry of Thiol-Disulfide Interchanges

RASTETTER, William H.
Massachusetts Institute of Technology
5 R01 CA 20574-05

Reactive Heterocycles--Cancer and Biomechanism

REICH, Edward
Rockefeller University
5 R01 CA 08290-16

Chemotherapeutic Deoxynucleosides and Other Agents

REINKE, Lester A.
University of Oklahoma
Health Sciences Center
7 R01 CA 30137-01

Influence of Ethanol on Carcinogen Activation

RICHARDSON, Arlan G.
Illinois State University
5 R01 CA 24856-02

Age-Related Changes in Chemical Carcinogenesis

RILEY, Edgar F.
University of Iowa
5 R01 CA 26511-02

Assay of Tumor Induction by X-Ray and Drug Modalities

ROMAN-FRANCO, Angel A.
University of Puerto Rico Medical Sciences
1 R01 CA 28894-01

Mechanism of Action of Carcinogenic Fibers

ROSSMAN, Toby G.
New York University
1 R01 CA 29258-01

Mutagenesis by Metals of Environmental Signficance

RUSSO, Jose
Michigan Cancer Foundation
5 R01 CA 23539-03

Studies of Mammary Gland Refractoriness to Cancer

SARDELLA, Dennis J.
Boston College
5 R01 CA 23454-03

Probing Carcinogens' Active Sites by F-Substitution

SCHAAP, A. Paul
Wayne State University
2 R01 CA 15874-07

Enzymatically Generated Singlet Oxygen in Carcinogenesis

SCHWARTZ, Arthur G.
Temple University
5 R01 CA 14661-08

Actions of Chemical Carcinogens on Cultured Cells

SCRIBNER, John D.
Pacific Northwest Research Foundation
5 R01 CA 23712-03

Early and Critical Events in Chemical Carcinogenesis

SHETTY, A. Subbayya
Florida Agricultural and Mechanical University
5 R01 CA 18817-03

Enzymatic Detoxification of Hydroxyanthranilic Acid

SIMENHOFF, Michael L.
Thomas Jefferson University
5 R01 CA 26571-02

In Vivo Nitrosamines and Cancer in Renal Failure

SIMS, Peter
University of London
2 R01 CA 21959-04

Mechanisms of Activation of Polycyclic Hydrocarbons

SINSHEIMER, Joseph E.
University of Michigan at Ann Arbor
5 R01 CA 25770-02

Epoxide Toxicity in Alkene Metabolism

SLAGA, Thomas J.
University of Tennessee Knoxville
5 R01 CA 20076-05

Polycyclic Hydrocarbon Metabolism and Binding in Skin

SOLT, Dennis B. Harvard University 1 R01 CA 28620-01	Sequential Analysis of Oral Carcinogenesis
SPECK, William T. Case Western Reserve University 2 R01 CA 23692-04	Potential Hazards of Phototherapy
STERNSON, Larry A. University of Kansas, Lawrence 1 R01 CA 28782-01	Chemical Characterization of Arylhydroxyl mines
STOMING, Terrance A. Medical College of Georgia 5 R01 CA 21481-05	Metabolism of 3-Methylcholanthrene in Liver and Lung
SUN, Albert Y. University of Missouri, Columbia 5 R01 CA 26586-02	Chlorinated Water and Membrane Functions and Neoplasia
SUZUKI, Yasunosuke Mount Sinai School of Medicine 1 R01 CA 29432-01	Carcinogenic and Fibrogenic Effects of Zeolites
SUZUKI, Yasunosuke Mount Sinai School of Medicine 5 R01 CA 24311-02	Pathogenesis of Experimental Malignant Mesothelioma
TANNENBAUM, Steven R. Massachusetts Institute of Technology 5 R01 CA 26156-02	Carcinogenic Nitrosamines from Primary Amines
Taylor, K. Grant University of Louisville 5 R01 CA 22365-03	Radical Reactions of Mutagens and Carcinogens
THURMAN, Ronald G. University of North Carolina, Chapel Hill 2 R01 CA 23080-04	Pharmacology of Carcinogen Activation in Intact Cells
UNDERWOOD, Graham R. New York University 5 R01 CA 25073-02	Study of Ultimate Carcinogen fr m Aromatic Amines
VAN DUUREN, Benjamin L. New York University 5 R01 CA 24124-03	Carcinogenic Acylating Agents and Mode of Action
VOLLHARDT, K. Peter University of California, Berkeley 2 R01 CA 20713-04	Activated Mutagenic and Aromatic Hydrocarbons

WARSHAWSKY, David University of Cincinnati 5 ROl CA 23515-03	Pulmonary Metabolism of the N-Heterocyclic Aromatics
WEINKAM, Robert J. Purdue University West Lafayette 7 ROl CA 28631-01	Chemotherapeutic and Carcinogenic Methlating Agents
WHALEN, Dale L. University of Maryland Baltimore College Campus 5 ROl CA 17278-05	Kinetic Studies of Aryl Epoxide Reactions
WHEELER, Larry A. University of California Los Angeles 5 ROl CA 22249-03	Oral Flora and Carcinogenesis of Dental Therapeutics
WONG, Lan K. University of Pittsburgh 5 R23 CA 27928-02	A Comparative Metabolism Study of PAH Analogues
YANG, Nien-Chu C. University of Chicago 5 ROl CA 10220-12	Molecular Mechanisms of Mutagenesis and Carcinogenesis
YANG, Shen K. Uniformed Services University 1 ROl CA 29133-01A1	Metabolic Activations of Mono-methylbenz (A) Anthracenes

CHEMICAL AND PHYSICAL CARCINOGENESIS BRANCH

GRANTS ACTIVE DURING FY 81

CHEMOPREVENTION

Investigator/Institution/Grant Number	Title
AWASTHI, Yogesh C. University of Texas Medical Branch Galveston 1 R01 CA 27967-01	Mechanism of Anti-Carcinogenic Effect of Antioxidants
BERTRAM, John S. Roswell Park Memorial Institute 5 R01 CA 25484-03	Inhibition of In Vitro Transformation by Retinoids
BLACK, Homer S. Baylor College of Medicine 5 R01 CA 13464-09	Photochemical Reactions Related to Skin Cancer
BUEDING, Ernest Johns Hopkins University 5 R01 CA 18251-06	Protection Against Mutagenic Effects of Drugs
CHOPRA, Dharam P. Southern Research Institute 1 R01 CA 26696-01A1	Biology of Airway Epithelial Lesions
GIFFORD, George E. University of Florida 5 R01 CA 22183-02	Nutritional Aspects of Vitamin A and Cancer
HILL, Donald L. Southern Research Institute 5 R01 CA 26389-02	Disposition and Chemopreventive Activity of Retinoids
HILL, Donald L. Southern Research Institute 1 R01 CA 30604-01	Prevention of ENU-induced Brain Cancer by Retinoids
MEDINA, Daniel Baylor College of Medicine 2 R01 CA 11944-10A1	Biology of Mammary Preneoplasias
MEHTA, Rajendra G. IIT Research Institute 5 R01 CA 26030-02	Retinoids and Mammary Carcinogenesis
ONG, David E. Vanderbilt University 5 R01 CA 20850-05	Cancer and Vitamin A

SHKLAR, Gerald
Harvard University
5 R01 CA 23524-03

Oral Carcinogenesis, Vitamin A and Retinoids

STOHRER, Gerhard
Sloan-Kettering Institute for Cancer Research
5 R01 CA 22609-03

Purine Derivatives and Other Chemical Carcinogens

SULLIVAN, Paul D.
Ohio University Athens
5 R01 CA 22209-03

Antioxidants Interaction with Benzopyrene and Derivatives

WANG, Chih-Cheng
Southern Research Institute
1 R01 CA 26815-01

Biotransformation of Retinoids In Vitro

WATTENBERG, Lee W.
University of Minnesota of Minneapolis-St. Paul
5 R01 CA 09599-23

Microsomal Induction and Response to Carcinogens

WATTENBERG, Lee W.
University of Minnesota of Minneapolis-St. Paul
5 R01 CA 14146-09

Inhibition of Chemical Carcinogenesis

WOLF, George D.
Massachusetts Institute of Technology
2 R01 CA 13792-04

Vitamin A and Glycoproteins of Skin Tumors

CHEMICAL AND PHYSICAL CARCINOGENESIS BRANCH

GRANTS ACTIVE DURING FY 81

MOLECULAR CARCINOGENESIS

Investigator/Institution/Grant Number	Title
ADAIR, Gerald M. University of Texas System Cancer Center 1 R01 CA 28711-01A1	Expression of Genetic Variation in Cultured Cells
ANDREWS, Alan D. Columbia University 5 R23 CA 26894-02	DNA Repair Processes in Xeroderma Pigmentosum
AVADHANI, Narayan G. University of Pennsylvania 2 R01 CA 22762-04	Cellular and Molecular Targets of Chemical Carcinogens
BANERJEE, Mihir R. University of Nebraska, Lincoln 5 R01 CA 25304-02	Chemical Carcinogenesis Mammary Gland Organ Culture
BECKER, Frederick F. University of Texas System Cancer Center 1 R01 CA 28263-01	Chromosomal Proteins in Chemical Carcinogenesis
BECKER, Frederick F. University of Texas System Cancer Center 5 R01 CA 20657-05	Phenotypic Analysis of Chemical Carcinogenesis
BERTRAM, John S. Roswell Park Memorial Institute 5 R01 CA 18197-05	Mechanisms of Carcinogenesis in Cell Culture
BHARGAVA, Madhu M. Yeshiva University 5 R01 CA 24842-03	Ligandin: Structure, Regulation, Role in Hepatocarcinoma
BLOOM, Stephen E. Cornell University, Ithaca 1 R01 CA 28953-01	Chick Embryos for Detecting Environmental Mutagens
BLUMER, Jeffrey L. Case Western Reserve University 1 R23 CA 30067-01	Lymphocyte Carcinogen Metabolism in Acute Leukemia
BOUCK, Noel P. Northwestern University 5 R01 CA 27306-02	Genetic Analysis of Malignant Transformation

BOURNE, Henry R. University of California, San Francisco 5 ROl CA 23218-03	Carcinogen Testing: A Mouse Lymphoma Mutation System
BOWDEN, George T. University of Arizona 5 ROl CA 26972-02	Postreplication Repair in Cultured Mammalian Cells
BOX, Harold C. Roswell Park Memorial Institute 1 ROl CA 29425-01	Molecular Studies of Carcinogenesis and Mutagenesis
BOYNTON, Alton L. National Research Council of Canada 1 ROl CA 28340-01	Assays for, and Actions of, Carcinogens and Promoters
BRESNICK, Edward University of Vermont 2 ROl CA 23514-04	DNA Repair after Polycyclic Hydrocarbon Administration
BRESNICK, Edward Universiy of Vermont 5 ROl CA 21258-03	In Vivo-In Vitro Model for Identification of Carcinogens
BROOKES, Peter University of London 5 ROl CA 25807-02	Biological Result of Carcinogen Induced Damage to DNA
BROYDE, Suse B. New York University 1 ROl CA 28038-01A1	Carcinogen-DNA Adducts: Linkage Site and Conformation
BRUNI, Carlo University of Virginia, Charlottesville 5 ROl CA 22025-02	Electron Microscopy of Muscle Carcinogenesis
BRYAN, George T. University of Wisconsin, Madison 5 ROl CA 11946-11	Carcinogenicity and Metabolism of 5-Nitrofurans
CAMPBELL, T. Colin Cornell University, Ithaca 5 ROl CA 23913-03	Effect of Perinatal Imprinting on Adult Carcinogenesis
CHAMBERS, Robert W. New York University 2 ROl CA 16319-06	Mutagenesis and Carcinogenesis
CHEN, Lan Bo Sidney Farber Cancer Institute 1 ROl CA 29793-01	Chemical Carcinogenesis of Epithelial Cells

CLARKE, Richard H.
Boston University
2 R01 CA 17922-04A1

Carcinogen-DNA Complexes: Structure and Interactions

CLARKSON, Judith M.
University of Texas System Cancer Center
2 R01 CA 19281-04A1

Cell Cycle Related DNA Repair Mechanisms

COOKS, R. G.
Purdue University West Lafayette
5 R01 CA 23859-03

Identity and Locale of Alkylated Nucleosides in DNA

CORBETT, Michael D.
University of Miami
2 R01 CA 23492-04

Hydroxamic Acid Production in Microbial Ecosystems

COVEY, Douglas F.
Washington University
5 R01 CA 23582-04

Suicide Substrates: Cancer and Endocrine Applications

COX, Ray
University of Tennessee Center for Health Sciences
5 R01 CA 15189-08

DNA Repair and Chemical Carcinogenesis

DAVIDSON, Richard L.
Children's Hospital Medical Center
5 R01 CA 26195-02

Chemical Mutagenesis Mechanisms in Mammalian Cells

DE SOMBRE, Eugene R.
University of Chicago
5 R01 CA 21525-03

Studies of Mammary Tumor Peroxidase

DI MAYORCA, Giampiero
College of Medicine and Dentistry of New Jersey
2 R01 CA 25013-03

Molecular Mechanism of Chemical Carcinogenesis

DIEBOLD, Gerald J.
Brown University
1 R01 CA 29912-01

Optoacoustic Detection of Carcinogens

DUKER, Nahum J.
Temple University
5 R01 CA 24103-03

Pathology of Repair of Carcinogenic DNA Damage

DULBECCO, Renato
Salk Institute for Biological Studies
5 R01 CA 21993-03

Normal and Neoplastic Development of the Mammary Gland

EBEL, Richard E.
Virginia Polytechnic Institute and State University
5 R23 CA 24364-03

Membrane Control of Microsomal Cytochrome P-450

EISENSTADT, Eric Harvard University 5 R01 CA 26135-02	Mutagenic Specificity of Environmental Carcinogens
EVANS, Helen H. Case Western Reserve University 5 R01 CA 23427-03	Radiation Induced Mutagenesis and Carcinogenesis
FAHL, William E. Northwestern University 5 R01 CA 25189-02	Hydrocarbon, Carcinogenesis in Mouse and Human Cells
FARBER, Emmanuel University of Toronto 5 R01 CA 25094-02	A Short-Term In Vivo Assay for Carcinogens
FARBER, Emmanuel University of Toronto 5 R01 CA 21157-05	Pathogenesis of Liver Cancer Induced by Chemicals
FARBER, John L. Temple University 2 R01 CA 12073-09	Hepatocarcinogenesis: A Role for Liver Necrosis
FELDBERG, Ross S. Tufts University 5 R01 CA 19419-05	Nature and Repair of a New Form of DNA Damage
FIALA, Silvio E. Shepherd College 2 R01 CA 14084-08	The Role of Carcinogen in Nucleic Acid Metabolism
FINK, Gerald R. Cornell University Ithaca 5 R01 CA 23441-04	Chemical Carcinogens and Frameshift Mutation in Yeast
FRAENKEL-CONRAT, Beatrice University of California Berkeley 5 R01 CA 12316-11	Alkylation of Polynucleotides In Vitro and In Vivo
FRANKLIN, Michael R. University of Utah 2 R01 CA 15760-07	Modification of Procarcinogen Enzymatic Activation
FREEDMAN, Herbert A. Downstate Medical Center 1 R01 CA 29052-01	H-2 Locus and Local Tumorigenesis by Methylcholanthrene
FRIEDBERG, Errol C. Standford University 5 R01 CA 12428-10	DNA Repair and its Relationship to Carcinogenesis

GARRO, Anthony J. Mount Sinai School of Medicine 5 R01 CA 22354-03	Detecting Alkyl Halide Mutagenicity with Isolated DNA
GEACINTOV, Nicholas E. New York University 2 R01 CA 20851-04	Characterization of Carcinogen-Nucleic Acid Complexes
GESSNER, Teresa Roswell Park Memorial Institute 5 R01 CA 24127-04	Conjugations and Carcinogen Metabolism
GOLD, Barry I. University of Nebraska 1 R01 CA 29088-01A1	Activation and Transportation of Nitrosamines
GOLDFARB, Stanley University of Wisconsin Madison 5 R01 CA 15664-06	Cholesterol Metabolism in Hepatic Neoplasms
GOLDTHWAIT, David A. Case Western Reserve University 1 R01 CA 27528-01	Chemical Carcinogenesis and DNA Repair
GOLDTHWAIT, David A. Case Western Reserve University 5 R01 CA 18747-05	Repair of X-Irradiated DNA in Normal and Cancer Cells
GOODMAN, Jay I. Michigan State University 5 R01 CA 13344-07	Repair Synthesis of DNA in Precancerous Rat Liver
GRIFFIN, Martin J. Oklahoma Medical Research Foundation 5 R01 CA 24459-03	Role of Epoxide Hydrase in Chemical Carcinogenesis
GRISHAM, Joe W. University of North Carolina Chapel Hill 1 R01 CA 29323-01	Analysis of Tumor Progression in Liver Cells In Vitro
GRISHAM, Joe W. University of North Carolina Chapel Hill 5 R01 CA 24144-03	Toxicity in DNA Repair Deficient and Proficient Cells
GUDAS, Lorraine J. Sidney Farber Cancer Institute 1 R01 CA 27953-01	Genetics/DNA Precursor Metabolism, Mutagenesis, Repair
GUPTA, Ramesh C. Baylor College 1 R01 CA 30606-01	Reaction of Carcinogenic Aromatic Amines with DNA

HANKINSON, Oliver University of California Los Angeles 5 RO1 CA 28868-02	Carcinogen Activation and Screening in Variant Cells
HANNA, Patrick E. University of Minnesota of Minneapolis-St. Paul 5 RO1 CA 21659-03	Carcinogen Activation Via Acyl Transfer
HANNA, Patrick E. University of Minnesota of Minneapolis-St. Paul 5 RO1 CA 24427-03	Bioactivation of Arylhydroxamic Acids: Sar Studies
HARD, Gordon C. Temple University 5 RO1 CA 24216-03	Experimental Pathology of Renal Carcinogenesis
HARRINGTON, George W. Temple University 2 RO1 CA 18618-06	Electroanalytical Studies of N-Nitrosamines
HARTMAN, Philip E. Johns Hopkins University 5 RO1 CA 26328-02	Detection Systems for Mutagens & Carcinogens
HASELTINE, William A. Sidney Farber Cancer Institute 1 RO1 CA 29240-01	Complementation Group A Locus of Xeroderma Pigmentosum
HASELTINE, William A. Sidney Farber Cancer Institute 5 RO1 CA 26716-02	DNA Damage/Repair by Environmental Carcinogens/Mutagens
HENDERSON, Earl E. Temple University 5 RO1 CA 23999-03	Characterization of Unique Lymphoblastoid Cell Lines
HERCULES, Kathleen University of California Los Angeles 1 RO1 CA 28449-01A1	Probing DNA Repair with SV40 Virus and Mutant Cells
HERRIOTT, Roger M. Johns Hopkins University 5 RO1 CA 25167-03	Pyrolytic Products of Proteinous Foods as Mutagens
HITTELMAN, Walter N. University of Texas System Cancer Center 1 RO1 CA 27931-01	Molecular Basis of Chromosome Aberrations
HNILICA, Lubomir S. Vanderbilt University 5 RO1 CA 26412-02	Experimental Hepatocarcinogenesis

HOLLENBERG, Paul F.
Northwestern University
2 R01 CA 16954-06

Hemoprotein-Catalyzed Oxygenations of Carcinogens

HOLOUBEK, Viktor
University of Texas Medical Branch Galveston.
5 R01 CA 22559-03

Interference of Azocarcinogens with RNA Processing

HOWARD-FLANDERS, Paul
Yale University
5 R01 CA 26763-02

Excision Enzymes and the Repair of Damaged DNA

HUMAYUN, M. Zafri
College of Medicine and Dentistry of New Jersey
5 R01 CA 27735-02

Mutagenesis by Carcinogens: A Molecular Approach

HURWITZ, Jerard
Yeshiva University
5 R01 CA 21622-05

Carcinogens on Pro- and Eucaryotic DNA Replication

HYLEMON, Phillip B.
Virginia Commonwealth University
2 R01 CA 17747-07

Bile Acids and Large Bowel Carcinogenesis

JACOBS, Lois J.
University of Wisconsin Madison
7 R01 CA 30450-01

Quantitative Mutagenesis Studies in Human Fibroblasts

JACOBSON, Myron K.
North Texas State University
1 R01 CA 29357-01

Poly(ADP-Ribose) Metabolism in Xeroderma Pigmentosum

JACOBSON, Myron K.
North Texas State University
5 R01 CA 23994-04

Alteration of NAD Metabolism by Chemical Carcinogens

JEFCOATE, Colin R.
University of Wisconsin Madison
5 R01 CA 16265-07

DNA Modification by Polycylic Hydrocarbons

JOHNSON, Eric F.
Scripps Clinic and Research Foundation
5 R01 CA 24146-03

Determinants of Carcinogen Activation/Detoxification

KALLENBACH, Neville R.
University of Pennsylvania
5 R01 CA 24101-02

Specificity in Frameshift Mutagenesis

KAN, Lou-Sing
Johns Hopkins University
1 R01 CA 27111-01

Model Alkylated Decanucleotide DNA Helices

KAUFFMAN, Shirley L.
Downstate Medical Center
5 R01 CA 17569-06

Lung Preneoplastic Hyperplasia and Chemical Carcinogens

KAUFMAN, David G.
University of North Carolina Chapel Hill
5 R01 CA 20658-05

Chemical Carcinogenesis and Cell Proliferation

KENNEDY, Ann R.
Harvard University
5 R01 CA 22704-03

Radiation and Chemical In Vitro Malignant Transformation

KIM, Sung-Hou
University of California Berkeley
5 R01 CA 27454-02

Crystalline Complexes of RNA with Small Molecules

KIMBALL, Paul C.
Ohio State University
1 R01 CA 27106-01

Chemical Cocarcinogenesis in the Rat: Gene Activation

KING, Charles M.
Michigan Cancer Foundation
2 R01 CA 23386-04

Mechanistic Approaches to Carcinogenesis

KLEID, Dennis G.
SRI Internationl
5 R01 CA 21593-03

Mechanism of Carcinogen-Induced Frameshift Mutations

KOHEN, Elli
Papanicolaou Cancer Research Institute
5 R01 CA 21153-03

Intracellular Enzyme Kinetics and Carcinogens

KOREEDA, Masato
University of Michigan at Ann Arbor
5 R01 CA 25185-03

Studies of Arene Oxids and Their Analogues

KULESZ-MARTIN, Molly
New York State Department of Health/
Roswell Park Division
1 R01 CA 31101-01

Quantitative Carcinogenesis in Cultures Epithelial Cells

LAISHES, Brian A.
University of Wisconsin Madison
5 R01 CA 24818-03

Proliferation Control During Hepatocarcinogenesis

LARCOM, Lyndon L.
Clemson University
2 R01 CA 21479-04

Biological Effects of DNA-Protein Crosslinks

LEFFERT, Hyam L.
University of California San Diego
7 R01 CA 29540-01

Early Effects of an Hepathoprocarcinogen

LIEBERMAN, Michael W. Washington University 5 R01 CA 20513-05	Chemical Carcinogen-Induced DNA Repair in Human Cells
LIEHR, Joachim G. University of Texas Health Sciences Center Houston 5 R01 CA 27539-02	Mechanism of Estrogen-Induced Renal Carcinogenesis
LIPSKY, Michael M. University of Maryland at Baltimore 1 R01 CA 28951-01	Multi-Stage Renal Carcinogenesis in Rats
LITMAN, Gary W. Sloan-Kettering Institute for Cancer Research 5 R01 CA 24861-03	Interaction of Benzoapyrene with Nuclear Proteins
LOEB, Lawrence A. University of Washington 5 R01 CA 24998-03	Genetic Miscoding by Metals
LOMBARDI, Benito University of Pittsburgh 2 R01 CA 23449-04	Choline Deficiency, Oval Cells and Hepatocarcinogenesis
LONGNECKER, Daniel S. Dartmouth College 5 R01 CA 17843-06	Studies of DNA Change Induced by Pancreatic Carcinogens
LOWE, Nicholas J. University of California, Los Angeles 5 R01 CA 25970-02	UV Light and Epidermal Polyamine and DNA Synthesis
LOWER, William R. University of Missouri, Columbia 5 R01 CA 23387-02	Detection of Carcinogens with ZEA and Tradescantia
MAHER, Veronica M. Michigan State University 5 R01 CA 21247-05	Role of Mutagenesis in Chemical Carcinogenesis
MANGEL, Walter F. University of Illinois, Urbana-Champaign 5 R01 CA 25633-03	A New Assay for Transformed Cells
MARCHOK, Ann C. Oak Ridge National Laboratory 1 R01 CA 30529-01	Preneoplastic Markers in Specific Lesion Cells
MC CORMICK, J. Justin Michigan State University 2 R01 CA 21289-04A1	In Vitro Transformation of Human Cells by Carcinogens

MEEHAN, Thomas D.
Michigan Molecular Institute
5 R01 CA 25106-02

Specificity in BAP Diol Epoxide Covalent Binding to DNA

MICHALOPOULOS, George
Duke University
5 R01 CA 23398-03

In Vitro Studies in Chemical Hepatocarcinogenesis

MILO, George E.
Ohio State University
5 R01 CA 25907-02

Chemical Carcinogen Induced Neoplastic Transformation

MORTELMANS, Kristien E.
SRI International
5 R01 CA 26124-02

DNA Repair Studies in Xeroderma Pigmentosum Cells

MOSES, Harold L.
Mayo Foundation
5 R01 CA 16816-06

Mechanism of Chemical Carcinogenesis In Vitro

MURRAY, Michael L.
Louisiana State University
Medical Center New Orleans
5 R01 CA 26355-02

Mediation of Nitrous Acid Mutagenesis by Polyamines

NAKANISHI, Koji
Columbia University
5 R01 CA 11572-12

Structural and Bioorganic Studies of Bioactive Compounds

NINAN-ADANGAPURAM, Thamby
Erie Community College
5 R01 CA 18478-03

Host-Mediated Assay of Carcinogenic and Mutagenic Agents

OHLSSON-WILHELM, Betsy M.
University of Rochester
5 R01 CA 25731-02

Radiation Sensitive Haploid Frog Cells

OLIVE, Peggy L.
Johns Hopkins University
1 R01 CA 28793-01

Mutagenicity and DNA Damage Using Spheroids

OLSON, Jack W.
University of Kentucky
1 R01 CA 31099-01

Hepatocarcinogenesis and Ornithine Decarboxylase

OLSON, Wilma K.
Rutgers The State University New Brunswick
5 R01 CA 25981-02

Carcinogenesis by Hydrocarbons: A Molecular Approach

PEGG, Anthony E.
Pennsylvania State University
Hershey Medical Center
5 R01 CA 18137-06

Persistence of Alkylated DNA in Carcinogenesis

PIETRZYK, Donald J.
University of Iowa
5 ROI CA 18555-06

Separation and Determination of Carcinogens

PIETTE, Lawrence H.
University of Hawaii at Manoa
2 ROI CA 10977-16

ESR Studies of Biological Free Radical Mechanisms

PLANCK, Stephen R.
University of Arizona
1 R23 CA 30466-01

Enzymology of Mammalian DNA Replication and Repair

POTTER, Van R.
University of Wisconsin Madison
5 ROI CA 17334-05

Oncogeny as Blocked Ontogeny: Studies of Liver Cancer

RANDERRATH, Kurt
Baylor College of Medicine
5 ROI CA 25590-03

Effects of Carcinogens on Nucleolar DNA

RICH, Alexander
Massachusetts Institute of Technology
1 ROI CA 29753-01

Chemical Carcinogenesis and DNA Structure

ROGAN, Eleanor G.
University of Nebraska Medical Center
5 ROI CA 25176-02

Binding of Aromatic Hydrocarbons to Nucleic Acids

SARMA, D. S. R.
University of Toronto
2 ROI CA 23958-04

DNA Repair/Replication in Chemical Carcinogenesis

SCHAEFFER, Warren I.
University of Vermont
5 ROI CA 12056-09

Studies of In Vitro Carcinogenesis with Aflatoxin B1

SCHROEDER, Alice L.
Washington State University
5 ROI CA 26314-02

Post-Replication-Repair of DNA in Neurospora

SEDWICK, W. David
Duke University
1 ROI CA 31110-01

Antifolate-induced Misincorporation of UDR in Human Cell

SELL, Stewart
University of California San Diego
1 ROI CA 29368-01

Radioimmunoassay of Alphafetoprotein

SHANK, Ronald C.
University of California Irvine
5 ROI CA 21955-03

Quantitative Alkylation of Nucleic Acids by Carcinogens

SHIM, Sang C. Korea Advanced Institute of Science 2 ROl CA 21729-04A1	Photochemistry of 5,M-Dimethoxy-coumarin
SICILIANO, Michael J. University of Texas System Cancer Center 1 ROl CA 28909-01	Genetics of Chemical Carcinogenesis in Fish
SINCLAIR, Peter R. Dartmouth College 2 ROl CA 25012-03A2	Liver Cell Culture for Study of Carcinogen Activation
SIRICA, Alphonse E. University of Wisconsin, Madison 1 R23 CA 29401-01	Isolation of "Preneoplastic" Cell Populations
SIROVER, Michael A. Temple University 1 ROl CA 29414-01	Regulation of DNA Repair in Chemical Carcinogenesis
SMITH, Leland L. University of Texas Medical Branch Galveston 5 ROl CA 21617-03	Potentially Carcinogenic and Atherogenic Sterols
SMUCKLER, Edward A. University of California, San Francisco 5 ROl CA 21141-05	Pathology of Chemical Carcinogenesis
SMULSON, Mark E. Georgetown University 5 ROl CA 25344-02	Carcinogens and Chromatin Structure and Function
SONG, Pill-Soon Texas Tech University 5 ROl CA 13598-09	Skin-Sensitizing and Carcinogenic Furocoumarins
SOROF, Sam Institute for Cancer Research 5 ROl CA 05945-18	Macromolecules in Chemical Carcinogenesis
SOUKUP, Shirley W. Children's Hospital Medical Center 5 ROl CA 18588-05	Early Changes in Potential Brain Tumor Tissue
SPELSBERG, Thomas C. Mayo Foundation 5 ROl CA 22272-03	Carcinogen-Chromatin Interaction in Cultured Cells
STEINER, Sheldon M. University of Kentucky 5 ROl CA 25431-02	Carcinogen Screening in Epithelial Cells

STOCK, Leon M. University of Chicago 5 R01 CA 20049-03	Free Radical Reactions in Chemical Carcinogenesis
STOHRER, Gerhard Sloan-Kettering Institute for Cancer Research 5 R01 CA 22458-03	Metabolic and Chemical Studies of Carcinogenesis
TEEBOR, George W. New York University 5 R01 CA 16669-06	Reparability of Chemical Carcinogenic Damage to DNA
TESSMAN, Irwin Purdue University West Lafayette 2 R01 CA 22239-04	Effect of Ultraviolet Light on Cellular Processes
TOPAL, Michael D. University of North Carolina Chapel Hill 1 R01 CA 28632-01	Effects of Carcinogen Modification of DNA Precursors
VAN LANCKER, Julien L. University of California Los Angeles 5 R01 CA 14840-06	Role of Repair Enzymes in Pathogenesis of Liver Cancer
WALBORG, Earl F., Jr. University of Texas System Cancer Center 5 R01 CA 27377-02	Membrane Glycoproteins During Hepatocarcinogenesis
WALKER, Graham C. Massachusetts Institute of Technology 5 R01 CA 21615-05	Mutagenesis and Repair of DNA
WALLACE, Susan S. New York Medical College 5 R01 CA 24953-02	Radiation Repair in Drosophila Melanogaster
WHALEN, Dale L. University of Maryland Baltimore College Campus 5 R01 CA 26086-02	Kinetics of Alkylation of RNA Components by Epoxides
WHITLOCK, James P., Jr. Stanford University 5 R01 CA 24580-03	Chemical Carcinogens and Their Cellular Receptors
YAGER, James D., Jr. Dartmouth College 5 R01 CA 26274-02	Error-Phrone DNA Repair in Hepatocarcinogenesis
YANG, Chung S. College of Medicine and Dentistry of New Jersey 2 R01 CA 16788-07	Monoxygenase: Properties and Carcinogen Activation

YERGANIAN, George
Northeastern University
5 R01 CA 25772-03

Cell Type/Host Assay of Mutagen-Carcinogen Activity

YU, Fu-Li
Rockford School of Medicine
1 R01 CA 30093-01

Aflatoxin B1 and Nucleolar RNA Synthesis

ZARE, Richard N.
Stanford University
5 R01 CA 23156-04

Laser Fluorescence Analysis

CHEMICAL AND PHYSICAL CARCINOGENESIS BRANCH

GRANTS ACTIVE DURING FY 81

SPECIAL PROJECTS

Investigator/Institution/Grant Number	Title
ABUL-HAJJ, Yusuf J. University of Minnesota of Minneapolis-St. Paul 5 RO1 CA 22467-03	Effect of Mammary Tumors on Steroid Metabolism
AHMED, Khalil University of Minnesota of Minneapolis-St. Paul 5 RO1 CA 15062-07	Studies of Normal and Neoplastic Prostate
ALBERT, Roy E. New York University 5 PO1 CA 26724-02	Inhalation Carcinogenesis of Environmental Agents
ALBERTINI, Richard J. University of Vermont 1 RO1 CA 30688-01	Direct Mutagenicity Testing in Man
ANDERSON, Lucy M. Sloan-Kettering Institute for Cancer Research 5 RO1 CA 22498-03	Chemical Carcinogenesis in the Athymic Mouse
BAIRD, William McKenzie Purdue University 1 PO1 CA 30234-01	Molecular Mechanisms of Carcinogen-DNA Interactions
BANERJEE, Mihir R. University of Nebraska Lincoln 5 RO1 CA 11058-12	Hormones, Cellular Biosynthesis and Mammary Carcinogenesis
BARKA, Tibor Mount Sinai School of Medicine 5 RO1 CA 24023-03	Tumor Promoters, Growth and Differentiation
BARKA, Tibor D. Mount Sinai School of Medicine 5 RO1 CA 24005-02	Isoproterenol and Carcinogenesis
BAXTER, C. Stuart University of Cincinnati 5 RO1 CA 24022-02	Cell Culture Studies of Environmental Promoters

BELMAN, Sidney New York University 5 RO1 CA 18536-05	Role of Cyclic Nucleotides in Tumor Promotion
BENFIELD, John R. City of Hope National Medical Center 1 RO1 CA 29373-01	Hamster Model of Bronchogenic Lung Cancer
BENFIELD, John R. City of Hope National Medical Center 1 RO1 CA 28045-01	Esophageal and Pancreatic Carcinogenesis
BERENBLUM, Isaac Weizmann Institute of Science 2 RO1 CA 21088-04	New Approaches to Systemic Two-Stage Carcinogenesis
BLOCK, Ronald E. Papanicolaou Cancer Research Institute 5 RO1 CA 13056-07	Characterization of Biological Tissues by NMR
BLUMBERG, Peter M. Harvard University 2 RO1 CA 22895-04	Mechanism of Action of Phorbol Diesters In Vitro
BLUMBERG, Peter M. Harvard University 5 RO1 CA 22895-03	Mechanism of Action of Phorbol Diesters In Vitro
BOKKENHEUSER, Victor D. St. Luke's-Roosevelt Institute for Health Sciences 2 RO1 CA 25763-07	Bacteria and Steroid Metabolism
BRANSCOMB, Elbert Warren Lawrence Livermore National Laboratory 1 RO1 CA 30613-01	Somatic Point Mutation Monitoring in Rabbits and Humans
BRASITUS, Thomas A. Columbia University 1 RO1 CA 28040-01	Colonic Epithelial Cell Plasma Membranes
BRESNICK, Edward University of Vermont 1 RO1 CA 30670-01	Comparitive Studies with Hamster Trachea and Human Bronchus
BROOKS, Sam C. Wayne State University 5 RO1 CA 22828-03	Fate of Steroid Hormones in Breast Tumor Cells
BROWN, Clark E. New England Deaconess Hospital 5 RO1 CA 24893-02	Cancer of Rat Prostate: Its Induction and Prevention

BROWN, Clark E. New England Deaconess Hospital 5 RO1 CA 24893-03	Cancer of Rat Prostate: Its Induction and Prevention
BRUEGGEMEIER, Robert W. Ohio State University 1 RO1 CA 28578-01	Biotransformations of Estrogens and Cancer
CASPI, Eliahu Worcester Foundation for Experimental Biology 5 RO1 CA 16464-05	Iodinated Estrogens in Breast Cancer
COSTLOW, Mark E. St. Jude Children's Research Hospital 1 RO1 CA 25170-01	Prolactin Receptor Regulation in Cultured Mammary Cells
COSTLOW, Mark E. St. Jude Children's Research Hospital 5 RO1 CA 23956-02	Hormone Receptor Regulation in Mammary Tissue
COSTLOW, Mark E. St. Jude Children's Research Hospital 5 RO1 CA 23956-03	Hormone Receptor Regulation in Mammary Tissue
CURPHEY, Thomas John Dartmouth Medical School 1 RO1 CA 30650-01	Pancreas and Liver Carcinogen Metabolism in Three Species
DIAMOND, Leila Wistar Institute of Anatomy and Biology 5 PO1 CA 21778-03	Environmental Factors in the Induction of Cancer
DIAMOND, Leila Wistar Institute of Anatomy and Biology 2 RO1 CA 23413-04A1	Tumor Promotor and Cell Differentiation
DIAMOND, Leila The Wistar Institute 1 RO1 CA 30446-01	Hydrocarbon Activation by Human and Hamster Cells
DIAMOND, Leila Gordon Research Conferences 1 R13 CA 31175-01	Gordon Research Conference on Cancer, 1981
EBNER, Kurt E. University of Kansas College of Health Sciences and Hospital 5 RO1 CA 17478-06	Mammary and Liver Prolactin Receptors
EPSTEIN, Samuel S. University of Illinois Medical Center 5 RO1 CA 24959-03	Quantitative Carcinogenesis Projection Between Species

ESTENSEN, Richard D. University of Minnesota of Minneapolis-St. Paul 2 RO1 CA 22195-04	PMA--A Cocarcinogen as a Lymphocyte Mitogen
FIALA, Emerich S. American Health Foundation 1 RO1 CA 31012-01	Disposition of Hydrazines: Species and Strain Effects
FISHMAN, Jack Rockefeller University 2 PO1 CA 22795-04	Specialized Center for Cancer Endocrinology
FRANKEL, Fred R. University of Pennsylvania 5 RO1 CA 17301-06	Mammary Cancer and the Nuclear Estradiol Receptor
FRANTZ, Andrew G. Columbia University 5 RO1 CA 11704-10	Prolactin Studies
FREEMAN, Aaron E. Center for Neurologic Study 7 RO1 CA 30220-01	Organoid In Vitro Model of Liver Carcinogenesis
GALA, Richard R. Wayne State University 5 RO1 CA 21597-03	Prolactin--Adrenal Involvement in DMBA Mammary Tumors
GARTE, Seymour J. New York University 5 R23 CA 23806-02	Biochemical Mechanisms of Tumor Promoters
GORSKI, Jack University of Wisconsin Madison 5 RO1 CA 18110-06	Prolactin in Normal and Neoplastic Pituitary Tissue
GOULD, Michael N. Wisconsin Clinical Cancer Center 1 RO1 CA 30295-01	Human vs Rodent Mammary Mediated Mutagenesis Assay
GRANDJEAN, Carter J. Midwest Research Institute 5 RO1 CA 27934-02	Diallylnitrosamine Carcinogenesis: Species Differences
GRIFFITH, O. Hayes University of Oregon 5 RO1 CA 11695-12	Photoelectron Microscopy of Mammalian Membranes
GUENGERICH, F. Peter Vanderbilt University School of Medicine 1 RO1 CA 30907-01	Purified Human Enzymes and Carcinogen Metabolism

GURPIDE, Erlio Mount Sinai School of Medicine 5 R01 CA 15648-08	Steroid Dynamics in Human Endometrial Cancer
HAM, Richard G. University of Colorado at Boulder 1 R01 CA 30028-01	Defined Medium for Human Mammary Epithelial Cells
HAZLEWOOD, Carlton F. Baylor College of Medicine 5 R01 CA 21624-03	Role of Water in Cancer Cell Division
HICKS, Ruth Marian Middlesex Hospital Medical School 1 R01 CA 31082-01	Carcinogenesis in Human and Rat Bladder Tissues
HILL, Donald Lynch Southern Research Institute 1 R01 CA 30296-01	Carcinogen Metabolism in Sensitive and Resistant Species
HOLLANDER, Vincent P. Joint Diseases North General Hospital 5 R01 CA 26041-02	Endocrine Factors in the Development of Plasmacytoma
HOLLANDER, Vincent P. Joint Diseases North General Hospital 5 R01 CA 10064-19	Hormonally Sensitive Tumors
HOMBURGER, Freddy Bio-Research Institute 5 R01 CA 24696-02	Syrian Hamster Model of Pancreatic Carcinogenesis
HUGGINS, Charles B. University of Chicago 5 R01 CA 11603-12	Endocrinology of Experimental Leukemia
JENSEN, Elwood V. University of Chicago 5 R01 CA 02897-25	Steroids and Growth
KAIGHN, M. Edward Pasadena Foundation for Medical Research 5 R01 CA 25089-02	Culture and Carcinogenesis of Human Bladder Urothelium
KERR, Sylvia J. University of Colorado Health Sciences Center 2 R01 CA 12742-09A1	Study of Methylations in Neoplasia
KLEINBERG, David L. New York University 5 R01 CA 16149-06	The Role of Prolactin in Human Breast Cancer

KLEIN-SZANTO, Andres J. P.
Oak Ridge National Laboratory
1 RO1 CA 29556-01

Importance of Dark Cells in Skin Carcinogenesis

LEAVITT, Wendell W.
Worcester Foundation for Experimental Biology
5 RO1 CA 23362-03

Hormone Receptor Ontogeny and Cancer

LEE, Chung
Northwestern University
5 RO1 CA 14727-07

Growth and Regression in Rat Mammary Tumors

LEHRER, Robert I.
University of California,
1 RO1 CA 30526-01

Blood Cell Receptors for Tumor-Promoting Phorbol Esters

LEUNG, Benjamin S.
University of Minnesota of Minneapolis-St. Paul
5 RO1 CA 25998-03

Hormonal Interaction in Mammary Carcinoma

LI, Jonathan J.
University of Minnesota of Minneapolis-St. Paul
2 RO1 CA 22008-04

Estrogen Carcinogenicity and Hormone Dependent Tumors

LINDAHL, Ronald G.
University of Alabama in University
5 RO1 CA 21103-02

Gene-Enzyme Relationship of Liver Aldehyde Dehydrogenase

LING, Gilbert N.
Pennsylvania Hospital
2 RO1 CA 16301-06

Water in Cancer and in Normal Tissues

MACINDOE, John H.
University of Iowa
5 RO1 CA 24687-02

Metabolism in Estrogen and Androgen Breast Cancer Cells

MAGUN, Bruce E.
University of Arizona
1 RO1 CA 29290-01

Mechanisms of Tumor Promotion In Vivo and In Vitro

MARKLAND, Francis S., Jr.
University of Southern California
5 RO1 CA 22910-03

Characterization of Mammary Glucocorticoid Receptor

MARTIN, R. Russell
Baylor College of Medicine
5 RO1 CA 15784-06

Human Macrophages and Other Cells in Carcinogenesis

MC GRATH, Charles M.
Michigan Cancer Foundation
5 RO1 CA 25482-02

Hormonal Control of Metastasis

MC GUIRE, William L. University of Texas Health Sciences Center San Antonio 5 RO1 CA 11378-12	Mechanism of Hormonal Control of Mammary Carcinoma
MEITES, Joseph Michigan State University 5 RO1 CA 10771-14	Neuroendocrine Control of Mammary and Pituitary Tumors
MENDELSOHN, Naomi Mount Sinai School of Medicine 5 R23 CA 27154-02	Maturation-Dependent Responses of Myeloid Cells
MICHALOPOULOS, George K. Duke University Medical Center 1 RO1 CA 30241-01	Cell Culture and Transplantation of Human Hepatocytes
MILLER, James A. University of Wisconsin Madison 5 PO1 CA 22484-04	Biochemical Studies in Chemical Carcinogenesis
MILLER, Jon P. SRI International 1 RO1 CA 24588-01A1	Effects of Tumor Promoters on Protein Kinases
MINTON, John P. Ohio State University 5 RO1 CA 22892-03	Hormones and Breast Cancer Prostaglandin Biosynthesis
MIRVISH, Sidney S. University of Nebraska Medical Center 5 PO1 CA 25100-02	Studies on N-Nitroso Compounds
OFNER, Peter Tufts University 1 RO1 CA 29513-01	Androgens in Prostatic and Epididymal Culture
OFNER, Peter Tufts University 5 RO1 CA 15776-06	Prostatic Differentiation and Sex Hormone Metabolism
PARDEE, Arthur B. Sidney Farber Cancer Institute 5 PO1 CA 22427-04	Molecular Analysis of Malignant Transformation
PARSA, Ismail State University of New York Downstate Medical Center 1 RO1 CA 30354-01	Interspecies Comparisons of Pancreas Carcinogenesis
PARSONS, Donald F. New York State Department of Health 1 RO1 CA 29255-01	Squamous Cell Carcinoma--Invasion Mechanisms

PURDY, Robert H.
Southwest Foundation for Research and Education
5 R01 CA 24629-03

Mutagenic and Carcinogenic Potential of Estrogens

RAO, Bantwal R.
Washington University
5 R01 CA 23579-03

Carcinogen Induced Changes in Steroid Hormone Action

RIVERA, Evelyn M.
Michigan State University
5 R01 CA 17862-05

The Biology of Rat Mammary Hyperplasias

ROSE, David P.
University of Wisconsin Madison
5 R01 CA 17579-06

Pituitary and Thyroid Function in Breast Cancer

ROSE, Leslie I.
Hahnemann Medical College and Hospital of Philadelphia
5 R01 CA 18737-06

Metabolic Studies of Human Uterine Neoplasms

ROSEN, Jeffrey M.
Baylor College of Medicine
5 R01 CA 16303-06

Hormonal Regulation of Breast Cancer

SAXENA, Brij B.
Cornell University Medical Center
5 R01 CA 13908-06

Gonadotropin Receptors

SCHECHTER, Joel E.
University of Southern California
5 R01 CA 21426-05

Rathke's Pouch-Derived Tumors: Effects of Hormones

SCHUT, Herman A. J.
Medical College of Ohio
1 R01 CA 30514-01

In Vitro Carcinogenesis Studies in Colon and Esophagus

SCOTT, Robert E.
Mayo Foundation
2 R01 CA 21722-04

Membrane Pathology in Carcinogenesis

SELKIRK, James K.
Oak Ridge National Laboratory
1 R01 CA 30355-01

Comparative Dynamics of Benzo(a)pyrene Metabolism

SIPERSTEIN, Marvin D.
University of California San Francisco
5 R01 CA 15979-08

Cholesterol Metabolism in Normal and Malignant Liver

STANLEY, Evan R.
Yeshiva University
5 R01 CA 21605-04

Hormone-Dependence and Autonomy in Tumor Growth

STUART, Robert K.
Johns Hopkins University
1 R01 CA 30491-01

Tumor Promoters and Regulation of Hematopoiesis

STONER, Gary D.
Medical College of Ohio at Toledo
1 R01 CA 28950-01

Carcinogenesis Studies in Cultured Rat Esophagus

STRAUSS, Bernard S.
Gordon Research Conferences
1 R13 CA 28113-01

Gordon Conference on Mutagenesis and Carcinogenesis

TANG, Frank Y.
University of Rochester
5 R01 CA 25455-03

Regulation of Mammary Gland Growth and Regression

TANNENBAUM, Steven R.
Massachusetts Institute of Technology
5 P01 CA 26731-02

Endogenous Nitrite Carcinogenesis in Man

TROSKO, James E.
Michigan State University
2 R01 CA 21104-04A1

Mutation and Derepression of Genes in Carcinogenesis

TS'O, Paul O.
Johns Hopkins University
1 R13 CA 27481-01

13th Jerusalem Symposium on Carcinogenesis

TS'O, Paul O.
Johns Hopkins University
5 P01 CA 16043-06

Biomedical Risks Caused by Nucleic Acid Perturbation

VESSELINOVITCH, Stan D.
University of Chicago
5 R01 CA 25549-03

Synthetic Steroids and Hepatocarcinogenesis

VESSELINOVITCH, Stan D.
University of Chicago
5 R01 CA 25522-03

Role of Sex Hormones in Hepatocarcinogenesis

VILLEE, Claude A.
Harvard University
5 R01 CA 24615-03

Hormone Induced and Dependent Tumors

WALKER, Bruce E.
Michigan State University
5 R01 CA 27535-02

Tertogenicity of Transplacental Des in Mice

WEBBER, Mukta M.
University of Colorado Health Sciences Center
1 R01 CA 28279-01

Human Prostatic Growth Regulation and Carcinogenesis

WEBER, George Indiana University-Purdue University at Indianapolis 5 P01 CA 13526-09	Correlated Study of Metabolic Regulation in Neoplasia
WEINSTEIN, I. Bernard Columbia University 5 P01 CA 21111-04	Molecular Events in Chemical Carcinogenesis
WEINSTEIN, I. Bernard Columbia University 5 R01 CA 26056-02	Cellular and Biochemical Effects of Tumor Promoters
WEISBURGER, John H. American Health Foundation 1 R01 CA 30658-01	Strain Differences in Carcinogenesis
WENDER, Paul A. Harvard University 1 R01 CA 28178-01	Synthetic Studies on Tumor Promoters and Inhibitors
WENNER, Charles E. Roswell Park Memorial Institute 5 R01 CA 13784-09	The Effect of Cocarcinogens on Cellular Membranes
WHITTAKER, J. Richard Wistar Institute of Anatomy and Biology 5 R01 CA 23394-03	Melanotic Expression in Carcinogen-Induced Melanomas
WILLIAMS, Jerry Randall George Washington University School of Medicine 1 R01 CA 31015-01	Mechanisms of Procarcinogenic Metabolism in Rat and Man
WOTIZ, Herbert H. Boston University 1 P01 CA 28856-01	The Role of Hormones and Binding Proteins in Cancer
WYNDER, Ernst L. American Health Foundation 5 P01 CA 12376-09	Environmental Carcinogenesis
YAGER, James D., Jr. Dartmouth College 5 R01 CA 23916-03	Role of Gonadal Steroids in Liver Carcinogenesis

CHEMICAL AND PHYSICAL CARCINOGENESIS BRANCH

GRANTS ACTIVE DURING FY 81

CREGS

Investigator/Institution/Grant Number	Title
ANDERS, Marion W. University of Minnesota 5 R01 CA 21899-03	Metabolism and Cellular Interactions of Haloforms
SAFE, Stephen H. University of Guelph 5 R01 CA 21814-03	Metabolism and Carcinogenicity of Haloaromatic Pollutants
WONG, John L. University of Louisville 5 R01 CA 16182-06	Molecular Mechanisms of Chemical Carcinogenesis

SUMMARY REPORT

SPECIAL PROGRAMS BRANCH

The Special Programs Branch (1) plans, develops, directs and manages a national extramural program of basic and applied research in the special emphasis areas of biometry, diet/nutrition, epidemiology, and preventive oncology as well as smoking and health; (2) establishes program priorities and evaluates program effectiveness; (3) provides a broad spectrum of information, advice and consultation to individual scientists and institutional science management officials relative to NIH and NCI funding as well as scientific review policies and procedures, preparation of grant applications, and choice of funding instruments; (4) provides NCI management with recommendations regarding funding needs, priorities and strategies for the support of relevant research areas consistent with the current state of development of individual research activities and the promise of new initiatives; (5) plans, develops and manages research resources necessary for the conduct of the coordinated research program; and (6) plans, organizes and conducts meetings and workshops to further program objectives, and maintains contact with the relevant scientific community to identify and evaluate new research trends relating to its program responsibilities.

The Special Programs Branch (SPB) was established February 11, 1979 by relocation of extramural efforts in diet/nutrition, epidemiology, and smoking and health from other areas within the Division of Cancer Cause and Prevention (DCCP), National Cancer Institute (NCI). Biometry was subsequently identified as a disciplinary area distinct from epidemiology. The programs were brought together in one branch to facilitate multidisciplinary approaches to research in these special areas of carcinogenesis. While the development of biometry has relevance to research in all of biology, it also contributes specifically to each of the SPB programs. Both Diet/Nutrition and Smoking and Health programs benefit directly from an interface with the Epidemiology Program which is, in turn, strengthened through efforts to better characterize studied populations by increasing use of laboratory determinations derived from research in diet/nutrition, smoking and health, as well as other areas of carcinogenesis. This symbiotic potential extends beyond the internal activities of SPB to the extramural community where interest in multidisciplinary efforts is increasingly evident. The newest SPB initiative, the Preventive Oncology Academic Award (POAA) Program, addresses this same need by providing an outstanding faculty member the opportunity to develop, coordinate, and expand varying research and educational programs potentially related to cancer prevention at the sponsoring institution.

Branch staff obtained permission to meet with an ad hoc advisory body for purposes of program planning and development of new initiatives. Although participants are selected for their interest and expertise in one or more of the research disciplines represented in the SPB, this group provides advice directly to the Board of Scientific Counselors (BSC), DCCP. Since the chairperson of the advisory body is also a member of the BSC, recommendations are transmitted directly back to the full membership of advisors to the Director, DCCP. At the first meeting held April 2-3, 1981, an in-depth review of existing programs was conducted, suggested revisions to the POAA were discussed, and recommendations for new initiatives in other program areas were made. It is anticipated that future concepts of need for new research efforts will be developed in this manner.

A brief description for each of the SPB Programs follows:

Biometry: This Program was established in late 1979 by transfer of approximately 25 percent of the research activity from the older, established Epidemiology Program. At present it remains a research grant activity, although contracts and/or interagency agreements are being developed to provide resources to the scientific community. This Program deals with mathematical models relevant to cancer biology, statistical techniques to test potential carcinogens, record linkage for investigations involving special population groups and cancer registries or death lists, cytogenetics and somatic cell genetics, techniques to evaluate cancer screening tests and procedures, evaluating estimates of cancer risk from low-dose exposure to carcinogens, and determining effects of patient characteristics on survival analysis or competing risks. A "state-of-the-art" workshop on "Studies in Cytogenetics and Somatic Cell Genetics Related to Cancer" is planned for late 1981.

Diet/Nutrition: The Diet Nutrition and Cancer Program (DNCP) was established in 1974 to "collect, analyze and disseminate information concerning the relationship between cancer and nutrition that would be beneficial in the prevention, diagnosis and treatment of cancer." This activity was supported entirely through use of the contract mechanism. In view of the increasing importance given to nutrition in all phases of the Cancer Program, the DNCP was reorganized in 1978 and specific elements were divided among three divisions and the Office of the Director (OD, NCI). Overall coordination and dissemination responsibilities were transferred from the OD, NCI to the Division of Resources, Centers and Community Activities (DRCCA), NCI in 1981. Funding and management activities continue to reside in the Division of Cancer Treatment (DCT), DRCCA, and the DCCP. All DCCP nutrition-related research activities are concerned with etiology and/or prevention and both grants and contracts are the responsibility of the Diet/Nutrition Program, Special Programs Branch (DNP-SPB). Following the NCI-wide program announcement in nutrition, the first investigator-initiated activities were funded in 1979. Another DNCP program announcement was issued in 1980 encouraging studies of alcohol and cancer. During FY'81 the DNP-SPB met with an advisory body to discuss the "significance of diets in animal carcinogenesis studies." It is anticipated that the resulting recommendations will be made available to investigators by the end of 1981. Two "state-of-the-art" workshops are planned for 1981 on (1) mutagens/carcinogens in cooked/processed foods, and (2) natural inhibitors of carcinogenesis in foods. It is anticipated that two or more Requests for Applications (RFAs) will be issued in late 1982 to encourage more investigator-initiated research in these areas.

Epidemiology: Since 1967 the extramural Epidemiology Program has consisted of five components: traditional epidemiology, human genetics, biomedical communications, behavioral research, and biometry. In 1973, the Division of Cancer Control and Rehabilitation was established with strong program interests in motivation, communication, and coping as behavioral problems. This led to some narrowing of Epidemiology Program activities in behavioral research, but with increased efforts related to etiology and prevention. During 1979, biometry (including human genetics) was identified as a component deserving special attention, leading to its development as a

new program within the Special Programs Branch. Areas of specific research interest in epidemiology currently supported entirely by the grants mechanism include natural history of neoplasia in humans, incidence and prevalence of various human cancer in different geographic locations, causal factors/causal associations related to cancer, intrinsic and extrinsic risk factors for various cancers, potential for preventive intervention, improved design and conduct of epidemiologic studies, and assessment of relative contributions of extrinsic and intrinsic risk factors. Plans are being developed to issue a specific request for descriptive epidemiologic studies of rare tumors in order to stimulate investigator-initiated research proposals in areas which tend to be understudied, but which may provide important insight into carcinogenic processes. Awards for meritorious research proposals in response to this RFA are planned for FY 1982.

Preventive Oncology Academic Award (POAA): The initial review for the first responses to this new program was conducted by the Cancer Research Manpower Review Committee, Division of Extramural Activities, NCI on June 5-7, 1980. Eight of nineteen POAA proposals were funded in late FY 1980 following mail ballot review by the National Cancer Advisory Board.

From its inception, a major strength of the POAA has involved the open recognition that institutional programs, like people, may vary substantially in their level of development. Some, with a stronger tradition in preventive medicine may be nearer the stage where formal implementation of courses in preventive oncology represents a primary thrust; most appear to require the opportunity provided by the POAA to allow the single most suitable candidate (determined by joint agreement among involved Deans, Dept. Chairmen, etc.) the opportunity to explore and facilitate development of in-depth ongoing research as well as potential new interdisciplinary research areas. The POAA has not been primarily concerned with formal course development, but rather sought to provide an appropriate institutional mileau among established scientists and educators in which such development could reasonably be expected. A more immediate POAA goal lies with providing outstanding students an opportunity for strong "hands-on" introduction to prevention-related research disciplines during a time period before their career decisions are finalized.

During the first two reviews, it became apparent that more detail on the qualifications of the candidate and opportunity for attaining additional educational objectives was desirable. As a result, the original POAA announcement was withdrawn on March 6, 1981. A draft revised Program Announcement was prepared for review and recommendations by an ad hoc advisory body to the Board of Scientific Counselors (BSC), DCCP. It is anticipated that a revised announcement will be reissued later in 1981.

Smoking and Health: The NCI Smoking and Health Program (SHP) was begun in 1968 solely as a contract effort. Since its relocation from the OD, DCCP to the Chemical and Physical Carcinogenesis Branch in 1978 and subsequent transfer to SPB in 1979, the SHP has sought to identify groups of individuals at high risk to tobacco-related diseases, to develop supplementary aids for assisting in smoking cessation, and for means by which to reduce the hazards of smoking products for persons unable to give up their habit or addiction. In a manner similiar to that described above for Diet/Nutrition,

the SHP activities have been divided between two NCI Divisions (DRCCA and DCCP) with coordination responsibilities transferred in 1981 from OD, NCI to DRCCA. The larger effort now termed Smoking, Cancer and Health Program/ NCI (SCHP/NCI) includes those tobacco related research activities in epidemiology and toxicology of the Special Programs Branch (SHP-SPB). Response to the FY'81 SCHP/NCI Program Announcement for investigator-initiated research (grant) proposals has been less than anticipated, although several meritorious proposals, including an epidemiologic study of smoking in relation to hepatocellular carcinoma and a multidisciplinary program project on tobacco-specific nitrosamines, have been awarded. Several RFAs or RFPs on smoker compensation in relation to changing cigarettes and in-depth studies of nicotine are contemplated for late 1981 with funding of meritorious proposals during FY 1982. Two "state-of-the-art" workshops on (1) passive/sidestream smoke and (2) biological response indicators and smoke component metabolites are planned for late 1981.

SPECIAL PROGRAMS BRANCH

FY 1981 Funds*

PROGRAM	GRANTS Number R01	R13	R23	P01	K07	TOTAL	GRANTS Dollars (in Millions)	CONTRACTS Number	CONTRACTS Dollars (in Millions)	SPB BRANCH Total Dollars (in Millions)
Biometry	30	1	1	2	0	34	4.65	0	0.00	4.65
Diet/ Nutrition	43	1	0	4	0	48	6.30	6	0.25 [0.37]	6.55
Epidemiology	40	2	3	3	0	48	7.49	[1]**	[2.50]	7.49
Smoking & Health	3	0	0	1	0	4	0.59	16	1.81	2.40
Preventive Oncology	0	0	0	0	1 [8]	1	0.05 [0.46]	0	0.00	0.05
TOTALS	116	4	4	10	1	135	19.08	22	2.06	21.14

*Estimated Total Dollars as of June 1981.
**[ ] Funded Late in FY 1980.

BIOMETRY SUMMARY

Description

The extramural Biometry Program provides support for 32 grants in basic developmental statistics areas unique to the conduct and analyses of laboratory/epidemiologic carcinogenic research. Approximately 40 percent of these grants involve theoretical statistics and mathematical modeling. Some of these deal with exploration of existing statistical methodologies to assess their appropriateness for analyses of clinical trials, survival and/or censured data, and dose-response and competing risk phenomena. Others address sample size determination in diverse situations and methods of treatment evaluation. Existing analytical methods are modified or extended when feasible and new methodologies developed when necessary. Mathematical models are being developed and tested in an attempt to relate cancer to cell biology and to explain gene-environment mechanisms by which cancer may be induced and/or altered. End products of this work should promote the design and analyses of better clinical trials, provide insight into the nature of the carcinogenic process, lead to new or more effective therapy, and contribute to cost effectiveness of screening programs.

Another 40 percent of the grants are genetically oriented. Some of these are strictly pedigree studies, while others incorporate new or refined methods for the identification of biological markers. Data bases resulting from these studies should give oncologists a powerful tool for distinguishing persons at high risk of cancer and, by so doing, lead to more effective cancer control programs.

Among the remaining 20 percent of the grants are two large multidisciplinary projects inherited from Research Resources Grants for Biomedical Computing. Initially (prior to 1971), these were designed to give mathematical support to research anywhere in their respective parent institutions. These have since become the sole responsibility of NCI and are now devoted to problem solving in areas of cancer research. Also among these 20 percent are several projects where data acquisition is a major feature. These particular projects came to the Biometry Program because of the complex computer software development necessary for file construction. Finally, there are two conference grants; one to promote standardized methods of data collection in tumor registries, and the other to disseminate modern oncologic statistical methodologies to statisticians involved (but not necessarily well trained) in cancer research.

The progress of the Biometry Program is difficult to assess in terms of recent productivity. It is a new program growing at a rate consistent with quality work. At its inception in 1979, the program was made up of 16 grants originally housed in the Epidemiology Program. Of these 16 grants, half were nearing termination either by investigator choice or recommendation of peer review. Hence, 75 percent of the program activities have come into existence within the past two years. Nevertheless, the previous scientific achievements of the investigators involved in these new activities indicates the potential viability of the program to the Special Programs Branch and, more importantly, the scientific community at large.

Research Accomplishments

In most medical experiments evaluating new treatments there is a "control" treatment to which new treatments must be compared. Experiments must be run in such a way that accurate answers are obtained, budget constraints adhered to, and unduly large numbers of patients not subjected to treatments inferior to the control treatment. Dudewicz, et. al., have approached this problem by developing a two-stage sequential model for treatment comparisons which, while allowing for heteroscedasticity (unequal variances), provides a convincing test of the null hypothesis (Amer, 1980). Work is now in progress to answer the next question: 'what proportion of subjects should be assigned to the "control" in order to optimize the power of the procedure?' The potential for use of these procedures in medical evaluation is extensive. For example, in animal tumor studies (either with an induced tumor or a transplanted human tumor) two problems are of interest: (1) how long does the animal survive after getting a malignant tumor? and (2) how large does the tumor grow? For fast-die situations, survival is of interest and the question is 'how efficient are various treatments?' Here the Dudewicz, et. al, procedure could be applied via a logarithmic transformation of the data when the class of survival distributions can be assumed to be log normal (the usual case). In the slow-die situations, the animal is sacrificed and the volume of the tumor measured. Here, normality is a realistic model, and (because of the expense of animals) investigators should be able to achieve large savings via (1) a two-stage allocation of animals in proportion to first-stage variance instead of equal-allocation (of sample sizes) and (2) sequential elimination and early detection of superior and inferior treatments.

Moolgavkar and Knudson have elaborated on their model for carcinogenesis and predictions regarding human and experimental cancers (Moolgavkar, In Press). The model incorporates two features: (1) transition of target stem cells into cancer cells via an intermediate stage in two irreversible steps and (2) growth and differentiation of normal target and intermediate cells. When put into mathematical terms, the model can be fitted to age-specific incidence data on human cancers of both children and adults and can illuminate the relative importance of agents that affect transition rates, tissue growth, and differentiation. Moolgavkar has applied the model to cancer of the breast in females where, with appropriate modifications to incorporate the physiological responses of breast tissue to menarche and menopause, it was found to generate age-specific incidence curves that are in close agreement with those in six test populations (Moolgavkar, 1980).

Moolgavkar has analyzed mortality data arising from carcinomas of the lung, bladder, and pancreas, in England and Wales during the period 1941-1970, using a model designed for analysis of time trend data (Moolgavkar, In Press). He concludes that (1) differences in mortality rates in the two sexes are attributable to differences in smoking habits, (2) carcinoma of the pancreas has increased slowly in non-smokers, and (3) cancer of the bladder has decreased in non-smokers. These results suggest that industrial exposures have played only a minor role in the increasing rates associated with these particular cancers.

The proportional hazards (the hazard rate in one treatment group is a constant multiple of the corresponding rate in the other group) regression model is used extensively to analyze the effects of covariates such as age, sex, and treatment

on patient survival. Shoenfeld has developed methods for testing this model. Three approaches have been taken. The first requires computation of a Chi-squared goodness of fit statistic; the second expands the model by adding terms and testing their coefficients; and the third is graphical (Schoenfeld, 1980). In the latter, a residual to the model equal to a specific covariate minus the expected value of that covariate given that an event (death) occurs at a given time, and where a specified set of patients are at risk at time (t - 0) is computed. Residual components are then plotted against time. These three approaches have been integrated into a single computer program.

Koziol, et al., have been investigating methods for extrapolating presumed carcinogenic risk from animal studies to human populations. Their method is novel in that it combines a dose-dependent carcinogenic hazard (determined from an animal experiment) with a human background time-dependent hazard (as calculated from incidence data) to arrive at an overall measure of risk of exposure to a carcinogen.

Another investigation involving animal experimentation as a means to learn more about how and why various cancers arise in man is that of Schneider, et al., (Moulton, In Press). The data are from population-based animal and human tumor registries functioning in the same geographic area. Studies are being made of cancer expression in man and his pet animal species living in the same geographic area. Certain factors peculiar to animals lend themselves to studying natural effects on cancer expression, which is not possible or not easy to study in man. Examples are sex hormonal effects (via neutering differences) and the nutritional-size effects (via the large size range in the dog species). The latter effect may help explain why many cancers have higher incidence in parts of the world where nutritional levels are highest.

A new algorithm, which is considerably faster than existing algorithms for calculating the exact significance level for r x c contingency tables has been developed by Pagano (Pagano, In Press). It uses a directed search technique that obviates the need for a total enumeration of the tables. Further savings are afforded by making use of the log-concavity of the hypergeometric function. Pagano has also developed and disseminated software for the non-parametric regression of survival analysis. This software is designed to handle large data sets, accommodates both time-varying and non-time-varying covariates and can be used to address problems of competing risk.

Projections

Future plans include continuation of research projects in the area of theoretical statistics with a strong emphasis on applications involving real cancer data. Less emphasis will be placed on uses of the basic computer systems which do not require extensive mathematical involvement and more on utilization of new computer hardware for graphics and image processing to further recent advances in statistical theory and mathematical/genetic modeling. Within the next year, certain of the genetic projects should reach a point where their methodologies/findings may be ready for preliminary presentation and/or publication. The Biometry Program itself is becoming a well-defined area in terms of interests and a recognized focal point for these disciplines within the NCI.

References:

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Pagano, M. and Taylor-Halverson, K.: An algorithm for finding the exact significance levels of r x c contingency tables. J. Amer. Statist. Assoc. (In press).

DIET AND NUTRITION SUMMARY

Description

The Diet and Nutrition Program of the Special Programs Branch (DNP-SPB) is responsible for those aspects of diet and nutrition research related to cause and/or prevention of cancer in humans, but does not include those parts of the NCI-wide Diet/Nutrition and Cancer Program (DNCP) dealing with special nutritional needs of sick patients or tumor-bearing animals. This DNP-SPB was initiated in February 1979 by transfer of 12 contracts and 18 ongoing grants from the Chemical and Physical Carcinogenesis Branch. Currently, there are six contracts and 48 grants in this program.

The DNP-SPB is interested in supporting laboratory investigations searching for etiologic factors related to diet and nutrition. These include mechanism studies of cancer induction by a variety of dietary constituents, such as fats of varying sources and saturation levels, proteins of various types and levels, fiber, nitroso compounds, mycotoxins, compounds associated with the gut including bile acids/fecal steroids and influence of fecal microflora. In addition, DNP promotes studies which focus on specific dietary factors, nutrients, or micronutrients, metabolic epidemiology, host factors involved in pathogenesis and development of methods or refinement of techniques for identifying putative carcinogens in foods, body fluids or feces, as well as influences of various methods of food processing and cooking.

Research Accomplishments

There is considerable epidemiological evidence that diet plays a role in the development of certain forms of cancer. On a prospective basis, an ongoing study is evaluating a cohort of approximately 120,000 women aged 30-55 in 1976. These women continue to provide detailed information on diet history, contraceptive practices, cigarette smoking, hair-dye use, and pertinent demographic and gynecologic histories. Outcome in terms of both morbidity and mortality is being determined over several years. The data collected will allow (a) assessment of the relationship of potential causal associations between breast cancer incidence and dietary components including total fat, linoleic acid, trans-fatty acids, saturated fats, cholesterol, and caffeine; (b) determination of possible protective associations between dietary components such as retinol, carotene, vitamins C and E, fiber, and breast cancer; (c) investigation of the relationship of potential causal associations between fat intake (total fat, animal fat, meat), use of processed foods, and the incidence of colon cancer; (d) determination of potential protective associations between intake of cruciferous vegetables, fiber, carotene, retinol and vitamin C in development of colon cancer; and (e) evaluation of potential protective association in smokers, between the intake of retinol, carotene, vitamins C and E, ingestion of cruciferous vegetables, and subsequent development of lung cancer. In addition, associations, either causal or protective, among the above dietary factors, artificial sweeteners and various forms of alcohol use can be made in relation to the incidence of ovarian, uterine and bladder cancers as well as melanoma.

An ongoing case-control study of Mormons living in Utah seeks comprehensive evaluation of the role and interrelationships of diet and nutrition to colon

cancer by correlating interview information with laboratory determinations of lipids and trace elements in the serum, steroids in the feces, and commercial as well as home-processed foods consumed by the study population. Case acquisition will be accomplished by the end of fiscal year 1981. A second part of this study involves experiments with rats using food specimens to determine the effect of various types of fiber on metabolism of toxicants.

Analysis of dietary information obtained from patients showed that high risk of cancer of the colon was unrelated to ingestion of meats or fats but was present in persons with low ingestion of cruciferous vegetables (Graham, 1978). The risk of both lung and bladder cancer was substantially reduced among smokers who ingested diets containing large quantities of vitamin A. In the case of bladder cancer, there was higher risk for heavy coffee drinkers (Mettlin, 1979). Investigations of the reliability of data obtained on diet in interviews of subjects as compared to their spouses indicated an acceptable degree of reliability (Marshall, 1980).

In a recently completed case-control study of women with cervical abnormalities identified through Pap smears, nutrient intake was estimated from computer analysis of three-day food records and 24-hour recall for 169 study participants (87 cases, 82 controls), including a subset of 49 pairs matched for age, race and parity. Younger age, greater frequency of sexual intercourse and younger age at first intercourse were associated with higher risk of cervical dysplasia. Multiple logistic analyses revealed that low vitamin C intake was an independent contributor to risk of developing severe cervical dysplasia when sexual activity variables were controlled (Romney, 1980).

A recently initiated case-control study of laryngeal and hypopharyngeal cancer will evaluate the relative carcinogenicity of various alcoholic beverages and their etiologic role among tobacco smokers and non-smokers in cancer of these two sites. Another study will evaluate the influence of alcohol on the development of intestinal tumors in rats induced by azoxymethane, intestinal bacterial flora, fecal bile acids, and neutral sterol.

Changes in body composition of rats bearing transplanted Morris 7800 hepatoma and receiving measured amounts of a semipurified casein diet suggest that physical exercise inhibits tumor growth by enhancing the competition for nutrients between host and tumor (De Rosa, 1980). Food restriction started very early in life (three to four weeks in rodents) increases maximal survivorship, reduces mortality rates and inhibits both cancer and other late-life diseases and immunologic aging. Preliminary studies indicate that food restriction in adulthood may also produce similar effects.

Several studies are underway to evaluate the influence of selected nutritional stresses, e.g., amino acid deficiencies, and high tryptophan diet on tumorigenesis due to chemical carcinogens. In one study, the combined feeding of phenobarbitol and a choline-devoid diet had a synergistic effect in promoting the emergence of foci of gamma glutamyltranspeptidase-positive hepatocytes in the liver of rats treated with a single dose of diethylnitrosamine (Shinozuka, 1980).

Certain investigators are currently studying the influence of the amount and degree of unsaturation of dietary fat on tumor production by chemical carcinogens. A preliminary observation in rats indicated that diets high in corn

oil enhanced mammary tumor development, but lard was not effective under these experimental conditions. High fat diets accelerated sexual maturation, as measured by age at vaginal opening, and may be responsible for alteration of the mammary gland response to dimethylbenz(a)anthracene. In another study, a high fat (HF) diet (33 percent corn oil) increased the susceptibility of the mammary gland to carcinogenesis. A change of diet from HF to low fat (LF) (five percent corn oil) reduced mammary tumors, whereas a change from LF to HF increased tumor incidence. In addition, enhanced susceptibility of the mammary gland to nitrosomethylurea carcinogenesis was observed in rats fed a HF diet before carcinogen treatment compared to that in rats fed a LF diet. The data also suggested that the effect of a HF diet required more than four weeks to manifest (Chan, 1981).

Semipurified casein-based diets varying in fat--10, 12, or 40 percent of calories and protein--8, 16, or 33 percent of calories--were fed to rats in order to determine how the dietary concentrations of these two major nutrient classes interact to influence mammary cancer. A linear increase in tumor incidence occurred with logarithmic increases in fat intake during the promotion phase of carcinogenesis (Clinton, 1981).

The rainbow trout is currently being used to study the effect of dietary protein on hepatocellular carcinoma induced by Aflatoxin B_1 (AFB_1). Trout treated with AFB_1 at the embryo stage developed more tumors when fed higher levels of protein, suggesting that the protein influenced the promotion of previously initiated tumors (Hendricks, 1981). Cyclopropenoid fatty acids (CPFA) occurring in cottonseed oil impair reproduction in rats and chickens, alter the fatty acid composition in the body lipids of several species and cause characteristic alterations in trout and rabbit liver parenchymal cells. Preliminary studies have demonstrated that CPFA act as co-carcinogens with aflatoxin B_1 and as carcinogens in the induction of liver cancer in rainbow trout (Bailey, 1979). Further studies are needed to understand the mechanisms of induction and promotion of cancer and concommitant physiological effects seen with dietary CPFA.

Several studies are underway to determine the role of selenium in carcinogenesis. They include determining the effects of selenium on chemically induced tumors in animals and the influence of selenium on lipid peroxidation. Neutron activation analysis technique for determining the three stable isotopes ^{75}Se, ^{76}Se and ^{80}Se in plasma, urine, and feces have been developed. This will enable the conduct of double isotope labeling experiments necessary to understand exchangeability of dietary pools of selenium and allow trace experiments in humans.

Dietary zinc deficiency in rats appeared to increase the incidence and shorten lag time for esophageal tumor induction by methylbenzylnitrosamine (Fong, 1978). Alcohol or zinc deficiency had about equal potency, but the combination of alcohol and zinc deficiency had an additive effect on induction of esophageal cancer.

Under certain conditions vitamin E has been shown to protect against induction of tumors by carcinogens. It is also effective in preventing further development of tumor from implanted tumor cells. It is conceivable that high levels of dietary vitamin E may function by increasing the ability of the immune system to attack developing tumor cells (Corwin, 1980).

Protease inhibitors have been shown to inhibit the action of the tumor promoter phorbolmyristate-acetate (PMA) in two stage carcinogenesis experiments in mice. Sprague-Dawley rats previously exposed to X-rays, to induce breast cancer, were fed diets containing high concentrations of raw soybeans which are rich in protease inhibitors. These animals developed fewer tumors than rats fed a diet low in proteases (Purina lab chow) or a diet lacking in protease inhibitors (casein diet) (Troll, 1980).

Analytical methods are being developed for the determination of volatile and non-volatile N-nitrosamines in direct-fire dried foods. Factors influencing the formation of N-nitrosamines under these conditions, as well as the potential of different foods for N-nitrosation in a simulated gastric environment, are also being examined. The N-nitrosoamides decompose at temperatures above 100°C and therefore would not withstand the temperatures normally encountered in the pan frying of meat (Kakuda, 1980).

Several multidisciplinary program projects are examining various dietary and nutritional influences on mechanisms of carcinogenesis. In one, specific dietary components such as protein, carbohydrate, vitamin A, folacin, vitamin B_6, ethanol and dietary fiber are being evaluated as they impact on several neoplastic models, including aflatoxin-induced liver hepatoma, the Walker 256 carcinoma cell line in rats, and the lymphoid L-1210 cell line in mice. The carcinogenic processes being examined in these studies include carcinogen metabolism, cocarcinogenesis and promotional phases of neoplastic cell evolution and growth, carcinogen sequestration in the gastrointestinal tract and immunocompetence capacity as related to carcinogen exposure and subsequent neoplastic cell growth.

Another program project seeks to define the role of vitamin A and its synthetic derivatives in the modulation, mediation, prevention and treatment of cancer. The scope of the projects ranges from investigations of the isolated retinol and retinoic acid binding proteins through work with normal and tumor cells in culture to the use of vitamin A and the retinoids in clinical protocol with cancer patients.

A newly initiated multidisciplinary program will examine the biochemical effects of medium chain triglycerides as a test of saturated vs. unsaturated fats, using colon fragments to study tumor promotion and isolate and determine the structure of mutagenic substances formed in fried meats and nitrosated fish.

Projections

There is a great need for expanded efforts in "metabolic epidemiology" in order to better characterize the differences in dietary habits which may alter physiological patterns. The effects of nutrient interaction on carcinogenesis, which might involve vitamin C and selenium, polyunsaturated fatty acids, iron, protein, etc., need further exploration. There is a need for development of methods for evaluating lipid peroxidation which are both more quantitative and more specific than present methods, e.g., malondialdehyde formation, pentane or ethane evolution, etc.

Characterization of the bile salts, fecal steroids (including cholesterol), gut microbial flora, and various types of fiber are critical needs in colon cancer studies. In-depth studies of naturally occurring non-toxic foods having the

potential of carcinogenesis inhibition are desirable to better understand the rationale for primary prevention activity.

As a result of planned workshops, formal advertisements for investigators in well-focused areas may be initiated. The use of the grant mechanism is anticipated for the basic research studies.

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LIST OF CONTRACTS

DIET AND NUTRITION PROGRAM

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International Agency for Research on Cancer (NO1-CP-71048)	Etiology of Esophageal Cancer in the Caspian Littoral of Iran	1610
National Center for Toxicological Research, FDA/HHS (YO1-CP-80208)	Comparisons of Cereal-Based and Semipurified Diets in Interactions of Diet/Nutrition and the Use of Genetically Distinct Animal Strains in Carcinogenesis Studies	1611
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CONTRACT NARRATIVES

DIET AND NUTRITION PROGRAM

ILLINOIS, UNIVERSITY OF (NO1-CP-75899)

Title: Effect of Dietary Protein Type and Level on Carcinogenesis

Contractor's Project Director: Dr. Willard J. Visek

Project Officer (NCI): Dr. A. R. Patel

Objectives: The overall objective of this project is to evaluate the induction and progression of spontaneously and chemically induced tumors in mice fed diets containing 10, 20 or 40 percent soybean protein or casein. Four carcinogens--benz(a)pyrene (BP), 1, 2-dimethylhydrazine (DMH), 2-acetylaminofluorene (AAF), or dinitrosopiperazine (DNP)--were fed as a part of the diets.

Major Findings: Preliminary toxicity studies of six-week duration were conducted using five dose levels of each carcinogen to determine the maximum tolerated dose (MTD). The most reliable indicator of toxicity was weight gain, which showed a dose response relationship. Food intake yielded similar responses but was not as reliable in measuring toxicity. Organ weights gave an indication of toxicity only after the animal had lost considerable body weight. Dose levels for a five-month toxicity study which followed were selected using the six-week toxicity data. The purpose of the five-month toxicity study was to more appropriately select doses which could be fed for 20 months. Parameters followed were the same as for the six-week toxicity study plus histopathological examinations.

While the six-week and five-month toxicity studies were in progress, absorption of each of the carcinogens in C^{14}-labeled form was studied separately. The time required for maximum absorption, the most appropriate duration for the absorption studies and the influence of source or level of protein on absorption were determined. Over 80 percent of the radioactivity present in the organs analyzed and in the excreta was recovered within 24 hours. Male mice excreted less radioactivity in urine and retained more in the target tissues when compared to the females. More radioactivity was found in the urinary bladders of males than females, and the bladder to kidney and bladder to GI ratios were far higher in males than in females. Significant protein sources by level interactions were found both in the excretion and distribution of radioactivity in the males while the level of protein in the diets caused significant changes in carcinogen excreted and in the GI contents of the female mice.

Significance to Biomedical Research and the Program of the Institute: Diet influences incidence of certain cancers in man independently of geographic and genetic factors. It is known that total caloric intake influences the number of spontaneous cancers in rodents. Colon cancer in man is highly correlated with the intake of fat and analyses of food consumption data also show a high correlation with the type and amount of protein consumed. Knowledge of the individual contribution of major nutrients to overall cancer incidence is not known and needs careful study because popoulations with high protein intakes also have high intakes of fat and total calories. Studies are needed to determine if plant proteins and animal proteins cause differences in spontaneous

and chemically induced cancers when consumed in graded concentrations with all other dietary ingredients held constant. Since chemicals are a recognized cause of cancer, it is necessary to know how the level of dietary protein influences absorption, excretion, distribution and metabolism of chemicals with established carcinogenic potential and how these factors correlate with cancer induction. The present studies will provide data for choosing dietary concentrations of carcinogen which allow animals to survive during long-term feeding studies designed to determine cancer incidence.

Proposed Course: This contract will expire on September 29, 1981.

Date Contract Initiated: October 1, 1977.

Current Annual Level: 0.

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (NO1-CP-71048)

Title: Etiology of Esophageal Cancer in the Caspian Littoral of Iran

Contractor's Project Director: Dr. N. E. Day

Project Officer (NCI): Dr. Donald H. Luecke

Objectives: (1) To identify the etiologic factors associated with the variations in esophageal cancer incidence along the Caspian Littoral of Iran. (2) To test the hypothesis that a powerful carcinogen is contained in opium dross and which may act on the esophagus rendered susceptible by nutritional inadequacies.

Major Findings: In the past year, the following has been achieved:

1. Antipyrine half-life determinations have been performed in sequential saliva samples from 200 individuals from low and high incidence areas. Quantitative determination of morphine metabolites in the urine samples from 1,200 individuals are nearing completion, and the results expected later in 1981. Mutagenicity testing has been performed on urine samples from 100 individuals from low and high incidence areas. The joint statistical analysis of these three factors will be undertaken when the complete data are available.

2. Coding of cancer registry data. The IARC has received from Teheran details of the last two years of cancer registration, 1977 and 1978, the coding of which had been delayed by the political situation in Iran. We have also received detailed information on the population in the Caspian region from the 1976 Iran census. A full analysis in collaboration with our Iranian colleagues is planned in the early summer.

3. Results obtained from mutagenicity tests of extracts of opium dross samples collected in Iran revealed the presence of aromatic compounds which showed potent mutagenicity when activated by a rat liver extract (Hewer et al., 1978, Lancet, 494-496). Subsequent studies on crude opium showed that its combustion products are highly mutagenic, greatly exceeding that of the mutagenic activity of tobacco smoke condensate, whereas the non-pyrolyzed material showed no activity (Bartsch et al., Mutation Res., 1980, 76, 1-50). Pyrolysis of the

major pure constituents of opium, such as morphine, demonstrates that the pyrolytic products derived from pure morphine represent a major portion of the mutagenicity obtained from combustion of crude opium.

Significance to Biomedical Research and the Program of the Institute: The work underway in 1978 was designed to test the hypothesis that a powerful carcinogen contained in opium dross was acting on an esophagus rendered highly susceptible by specific nutritional inadequacies; despite recent logistic difficulties, the parts of this study which have been salvaged will provide some of the answers. This model may be of general significance in the etiology of esophageal cancer since a similar sequence of events but with different risk factors, may be operating in other regions where esophageal cancer is frequent. In particular among the black population of the United States, similar dietary deficiencies have been tentatively linked to esophageal cancer.

Proposed Course: This contract will expire on June 30, 1981.

Date Contract Initiated: June 30, 1977.

Current Annual Level: 0.

NATIONAL CENTER FOR TOXICOLOGICAL RESEARCH FDA/HHS (YO1-CP-80208)

Title: Comparisons of Cereal-Based and Semipurified Diets in Interactions of Diet/Nutrition and the use of Genetically Distinct Animal Strains in Carcinogenesis Studies.

Contractor's Project Director: Dr. Neil A. Littlefield

Project Officer (NCI): Dr. A. R. Patel

Objectives: To compare the effects of a semipurified diet and a cereal-based diet on the incidence of "spontaneous" pathological observations, estrogen (estradiol)-induced lesions, and on liver and bladder carcinogenic lesions induced by 2-acetylaminofluorene (2-AAF) in an inbred (BALB/c) and a hybrid (B6C3F1) mouse stock.

Major Findings: Food consumption and growth appeared within the normal range on both diets at all dose levels among mice in the subchronic portion of the 2-AAF study. Several mice on the semipurified diet died during the study. The causes of death have not been determined.

Preliminary evaluation of data from a study to evaluate the effects of different storage conditions on the diets indicated that the semipurified diet should always be stored under refrigeration. Exposure of this diet to elevated temperatures, such as those often encountered during shipping, could result in excessive deleterious alterations.

Significance to Biomedical Research and the Program of the Institute: The semipurified diet (AIN-76) holds promise for future use as a standard diet in carcinogenesis studies. Completion of this study will provide data relative to the usefulness of the AIN 76 diet, as well as the NIH 07 open formula diet, as

standardized diets for toxicity/carcinogenicity studies. The adoption of a standard diet with uniform contents from batch to batch should minimize the variability in results that may be influenced by diet. The use of a standard diet in protocols can lead to more accurate comparisons between experiments.

This experiment will provide a data base comparing the histopathology of control mice and the responsiveness of mice to chemical insult when maintained on a semipurified and a cereal-based diet. The development of this data base is important to DNCP since the diet is being evaluated for use in diet and cancer studies.

Proposed Course: Using results obtained from the short-term studies, lifetime studies will be conducted on mice over a 24 month-plus period.

Date Contract Initiated: September 30, 1978.

Current Annual Level: $250,000.

SMITHSONIAN INSTITUTION (NO1-CP-85631)

Title: Baseline Research Film Study Among the Inhabitants of Hunza in the Karakoram Mountains of Pakistan.

Contractor's Project Director: Dr. E. Richard Sorenson

Project Officer (NCI): Dr. A. R. Patel

Objectives: To obtain a comprehensive film record of characteristic Burusho behavior, including dietary habits in relation to the normal events of daily life, as a means of discovering the behavioral and dietary patterns which sustain their type of adaptation to the conditions of life provided by the harsh ecology of the Karakoram Mountains in Pakistan.

Major Findings: In anticipation of the beginning of the project, basic preparations were undertaken in June 1978. However, due to the political situation in Pakistan, the contractor was unable to obtain necessary permissions from the Government of Pakistan to begin field work.

Proposed Course: This contract will expire on May 21, 1981.

Date Contract Initiated: May 22, 1978.

Current Annual Level: 0.

SRI INTERNATIONAL (NO1-CP-85620)

Title: Validation and Standardization of In Vitro Techniques to Assess the Effects of Diet/Nutrition on the Mutagenic/Carcinogenic Potential of Human Secretions and Excretions.

Contractor's Project Director: Dr. J. H. Peters

Project Officer (NCI): Dr. A. R. Patel

Major Objectives: The major objectives of this multidisciplinary project are to (1) develop and refine techniques for determining the mutagenic potential of various foods and of the body fluids and excretions of subjects consuming these foods; (2) identify, separate and quantitate the compounds responsible for the mutagenic activity in foods and in the body fluids and excretions of subjects consuming these foods; (3) recommend standardized methodologies and techniques for performing the above tasks; (4) assess the applicability of these methodologies and techniques to large-scale screening; and (5) determine mean values and ranges for mutagenic potential of certain foods prepared in different ways and for the mutagenic potential of the body fluids and excretions of subjects consuming these foods.

Major Findings: Bibliographies on antioxidants, vitamins, and anti-inflammatory steroids as modifiers of carcinogenesis, fecal mutagens and carcinogens, and on tumor promoters in urine and feces has been compiled.

The protocol for screening human urine for mutagens and/or promutagens was extended to provide for a 1,000-fold concentration of urinary constituents. In an investigation of one of the possible complexities of screening human urine concentrates (HUCs), it was found that the mutagenic activities of 2-acetylaminofluorene (AAF) and of the promutagens of tryptophan pyrolysis, TRP-P-1 and -2, for Salmonella typhimurum strain TA98 and of 2-anthramine (AN) for strain TA100 were significantly enhanced by HUCs from either cigarette smokers or nonsmokers. The enhancement observed was dose-related either for amount of HUC or promutagen employed. Maximal enhancement of the mutagenic activity of AAF for TA989 was about 10-fold; maximal enhancement of AN for TA100 was about 20-fold. A protocol was developed for testing HUCs for enhancement using 500 ul of a 1,000-fold HUC and three graded amounts of HUC for each of three levels of AAF for TA98 and AN for TA100.

Through the cooperation of Dr. H. E. Sauberlich, Western Human Nutrition Research Center, USDA, at the Presidio of San Francisco, pooled urine samples from each of three weeks were obtained from 11 nonsmoking volunteers under strict dietary control. Concentrates (1,000-fold) were tested for mutagenic activity using strains TA98 and TA100 as specified in the standard protocol. None of the 33 samples exhibited mutagenic activity for either TA98 or TA100. However, they enhanced the mutagenic activity of promutagen samples.

The mutagenic activities of the tryptophan, the glutamic acid, and the soybean globulin pyrolysis promutagens, TRP-P-1 and -2, GLU-P-1 and -2, and GLOB-P-1 and -2, respectively, was determined. A liquid chromatographic technique was developed to resolve most of these compounds from one another and from harman and norharman. Detection employing fluorescence provided sensitivities for quantitation in the picogram range.

Significance to Biomedical Research and the Program of the Institute: Standardized, validated procedures are required for detecting mutagenic activity in human excreta to assess the risk of populations to various potential mutagens and carcinogens. The observation that urines, regardless of source, contain materials that enhance the activities of known promutagens suggest that positive findings of mutagenic activities in HUCs should be interpreted with extreme caution.

Proposed Course: This contract will expire on September 29, 1981.

Date Contract Initiated: September 30, 1978.

Current Annual Level: $243,252.

WASHINGTON UNIVERSITY (NO1-CP-85662)

Title: Validation and Standardization of In Vitro Techniques to Assess the Effect of Diet/Nutrition on the Mutagenic/Carcinogenic Potential of Human Secretions and Excretions

Contractor's Project Director: Dr. Barry Commoner

Project Officer (NCI): Dr. A. R. Patel

Objectives: The objectives in the third year of this project have been to (a) test the effectiveness of the proposed standard urine mutagen protocol for detecting low levels of mutagens in the urine of populations exposed to environmental sources of mutagens, (b) study alternative means of quantifying the mutagenic activity detected as in (a) above, (c) further analyze the interference of urinary toxins on the detection of mutagens, and (d) examine alternatives to the standard protocol to avoid the problems identified in (c).

Major Findings: No apparent increase in sensitivity is gained by testing urine extracts over a range of concentrations up to 100 ml. per plate. Using a range up to 50 ml. appears adequate. Thus, smaller urine samples can be used, an important logistical improvement in the protocol. The standard urine protocol proposed in the second year of contract work called for a two-step elution of the XAD-2 columns--first with methylene chloride, and then with acetone. Urine samples from a population exposed to a variety of mutagens (cigarette smoke and other unknown exposures) were analyzed using this protocol. Mutagenic activity appeared with approximately equal frequency in the methylene chloride and acetone fractions prepared from the samples. In a comparison with the protocol in which acetone alone is used for elution, it was found that the two-step elution resulted in fewer toxic samples.

Extracts of nonsmokers' urine enhance the mutagenic activity of the powerful mutagen 2-acetylaminofluorene, but do not enchance the activity of another mutagen, benzo-(a)-pyrene. This enhancement is due largely to constituents of the urine itself rather than to artifacts of the extraction process. Nonsmokers' urine extracts did not, however, enhance the activity of smokers urine extract.

Significant increases in the mutagenic activity detectable in a smoker's urine sample can be achieved by a basic wash of the eluate from the XAD-2 column. An in situ wash of the column itself following the application of the urine does not have the same effect.

The "bacterial fluctuation test" was used to analyze several smokers' urine samples. Mutagenic activity was detectable from smaller volumes of urine than in the standard plate test, suggesting that the fluctuation test may be a more sensitive way to detect low levels of mutagens in human urine.

Significance to Biomedical Research and the Program of the Institute: The urinary mutagen assay is becoming an important tool for studying environmental carcinogen exposure. However, before it can gain widespread acceptance, it must be capable of producing consistent, reliable results. The problems of interference and interpretation that this research addresses are what stand in the way of a reliable assay.

Proposed Course: This contract will expire on September 29, 1981.

Date Contract Initiated: September 30, 1978.

Current Annual Level: $122,745.

EPIDEMIOLOGY SUMMARY

Description

The Epidemiology Program encompasses studies utilizing basic descriptive and analytic, retrospective case/control, historical, and prospective cohort methodologies. The multifactorial nature of disease etiology is recognized in multidisciplinary approaches within the program. Causal associations of various cancers with occupational, environmental, genetic and/or familial and behavioral risk factors provide necessary information for structuring programs of prevention and intervention.

Of 46 research efforts funded this fiscal year, 39 are individual investigator-initiated grants (R01), three are multifactorial program project grants (P01), three are new investigator grants (R23), and one is a continuing conference grant (R13).

Twelve studies have terminated or will be completed during FY'81, and ten new studies were entered in the program through May, 1981. Case-control studies indicating relative risk of disease comprise 55 percent of the SPB Epidemiology Program. These are retrospective studies in which patient characteristics or exposure to specific contaminants or foods are evaluated in patients already diagnosed with cancer of a specific site. Cancer of the lung, heart, ovary, skin (malignant melanoma), brain (glioma), colon-rectum, lymphoma, multiple myeloma and leukemia were compared with characteristics of exposures of matched controls. Cohort studies, which comprised 25 percent of the grants, included follow-up of women receiving mammographic examinations as part of an ongoing breast cancer screening program, parents of children with genetic or congenital aberrations, long-term hepatitis B surface antigen carriers, residents of an area contaminated with low-level ionizing radiation (plutonium), and alcohol and cigarette users. Descriptive epidemiology studies which seek possible causal clues provided in incidence, prevalence, and mortality statistics comprise another 20 percent of the epidemiology grants.

The effect of life-style and exposures in relation to cancer is of growing interest. Two studies investigating malignant melanoma examine risks associated with exposures to sunlight and chemicals. Hypotheses involving viral associations or etiology continue to have a priority in 11 investigator-initiated studies. The viral associations include hepatitis B virus (HBV) and hepatocellular carcinoma; Epstein-Barr virus (EBV) and Hodgkin's disease (HD); Herpesvirus II and cervical cancer. Three studies of incidence and mortality explore the elevated risk of some cancers among farmers.

The importance of disentangling prenatal and childhood environmental exposures in the etiology of childhood cancers is receiving attention in three studies. In [illegible]ing studies investigators are collaborating to examine the long-range car[illegible]c hazard following radiotherapy and/or chemotherapy for Hodgkin's dis[illegible] further effects of radiation are addressed in a study which hypothesi[illegible] causative link between dental X-ray exposures and parotid gland tumors. Relationships of endogenous and exogenous hormones and female/male reproductive organs are the focus of research in 11 studies. While breast cancer continues

to be a major component in two of three program projects, these research efforts also include cancer of the pancreas, ovary, testes, Hodgkin's disease and multiple myeloma and non-Hodgkin's lymphoma. The remaining project is providing important epidemiologic information regarding the association between persistent hepatitis B infection and hepatocellular carcinoma.

One ongoing study utilizes a genetic marker to explore the cellular development and natural history of human neoplasms. Three new grants will document current implications of dietary intake of alcohol and the impact of cigarette smoking as a primary risk factor in oral and pharyngeal squamous cell carcinoma. Two studies, which deal with investigations of the potential protective effects, focus on lung cancer. One study examines the relationship of vitamin A and lung cancer; the other seeks information utilizing a population at low risk for lung cancer.

Research Accomplishments

The possible association of coffee consumption to pancreas cancer risk, now the fourth most common fatal malignancy in the United States, is of increasing research interest. A case-control interview study conducted on 369 patients with histologically diagnosed cancer of the pancreas and 644 control patients regarding use of tobacco, alcohol, tea, and coffee, found a weak positive association between pancreatic cancer and cigarette smoking. However, a stronger association was claimed for coffee consumption and pancreatic cancer evident in both sexes and not affected by controlling for cigarette smoking. The study yielded a dose-response relationship with relative risk of 1.8 associated with daily drinking of up to two cups of coffee per day and a relative risk of 2.7 for three or more cups (MacMahon, 1981).

In another study, there was no compelling evidence found to support any special environmental cause for cancer of the pancreas. This study sample of 1,885 males and 1,729 females with cancer of the exocrine pancreas involved a population diverse in demography and premorbid experience but relatively uniform in medical care, and was drawn over a six-year period from a population-based Cancer Surveillance Program which lists all cases in a county of approximately seven million people (Mack, 1981). A case-control study to further delineate possible effects has recently been initiated.

It remains controversial whether Hodgkin's disease (HD) represents a true malignancy or an inflammatory disease. Current research suggests the disease in young adults may be a reaction to a common viral infection, modified perhaps by late age at exposure (Gutensohn, 1980a). The most likely candidate appears to be Epstein-Barr virus (EBV) (Evans, 1980). The epidemiologic similarities between Hodgkin's disease in the young and paralytic poliomyelitis may be a rare consequence of a common infection, with increased risk associated with a history of infectious mononucleosis. Childhood social environment appears to be an important factor in influencing the risk of the disease in this age group (Gutensohn, 1980b). In a study of 225 HD cases, with diagnoses based on hospital records, and population controls matched for age, sex, and study area, risk in young adulthood (15-19 years) appeared to be associated with factors that delay or diminish exposure to infectious agents, higher social class, more education, small family size, and early birth order position. Such associations were not explicable by either radiation or chemical carcinogenesis, but were

consistent with a virus-induced pathogenesis, with risk increasing at age of infection (Gutensohn, 1980).

Reports of a recent increase in the incidence of metachronus or subsequent malignant neoplasms (SMN) among patients with Hodgkin's disease (HD) suggest an association with the trend toward agressive therapeutic management of HD. Actuarial probability curves reveal that the combination of radiation therapy and chemotherapy has significantly potentiated any intrinsic susceptibility of leukemogenesis in HD patients (Brody, 1980). The historical progression from non-intensive palliative therapy to intensive curative therapy has enabled an epidemiologic "natural experiment" which may allow investigators to characterize the specificity and strength of the association between exposure to ionizing radiation and/or chemotherapy and the SMN in HD.

Recent findings in an ongoing eight year breast cancer study indicated that manipulation of female hormonal milieu during or prior to menopause may be related to the subsequent estrogen receptor (ER) status of incident breast cancers. Patients with ER protein in breast cancer tissue have approximately 60 percent chance of responding to endocrine therapy; conversely, a response to endocrine therapy in the absence of ERs occurs in fewer than 10 percent of breast cancer patients. A history of prior total bilateral oophorectomy and exogenous estrogen use was elicited from 45 women with breast surgery for cancer, 19 whose neoplasms were ER-positive and 26-ER negative. In the ER-positive group, there was a statistically significant history of oophorectomy in 0/19 and estrogen in 3/19. In the ER-negative group, corresponding values were 8/26 and 12/26 (Wallace, 1980). The finding of low or absent ER in women with prior oophorectomy needs to be replicated in other studies. If confirmed, it may help explain differing ER-positive rates in various series of breast cancer patients, and it may suggest a separate category of breast cancer patients for analyses of various treatment modalities and prognosis.

It is generally recognized that age-standardized cancer incidence and mortality rates are higher for white women than black women in the United States. Racial differences in breast cancer risk factors may explain the less well-known fact that age-specific rates for young black women are higher than those for young white women. Cancer incidence rates were obtained from the Third National Cancer Survey (1969-1971) and a population based Cancer Surveillance Program for 1972-1976. The known risk factors for breast cancer (early menarche, late age at first full-term delivery and at late age of menopause) differed in black and white women. The observed difference in incidence might be explained by the earlier age at menarche among blacks which would increase risk of developing cancer at a younger age, but followed by an earlier first birth and earlier menopause (both of which are supported by known data) which would lower the risk of developing breast cancer later in life. The data presented suggest that the differences in rates between blacks and whites can be explained, at least qualitatively, by these risk factors. (Gray, 1980).

A case-control interview study was conducted among 185 women with microscopically confirmed intracranial meningioma identified through a population-based cancer surveillance program and 185 matched neighborhood controls to investigate possible causes of this cancer. Slightly over half of the 185 patients were over 50 years of age at the date of diagnosis; 17 percent of the pairs were black, 25 were Spanish-surnamed and 143 were other whites. No association was

found between meningioma occurrence and farm residence, or the occurrence of cancer or central nervous system tumors among relatives, or use of hair dyes or tints. Three primary factors appeared to be associated with meningioma occurrence: a history of head trauma, consumption of certain cured meats, and exposure to medical and dental X-rays to the head. For diagnostic X-rays, the strongest association was with early exposure (<20 years old) to full-mouth dental X-ray series. While findings on exposure to medical and dental radiation are consistent with the hypothesis of a causal association, poor recall of previous exposure makes such findings difficult to interpret. The association with early radiation exposure may represent a dose effect since full-mouth series with older X-ray units exposed the patient to up to 250R (Preston-Martin, 1980).

Mortality studies have indicated that farmers may have elevated risk for some cancers. Using proportionate mortality rates (PMRs), a recently completed descriptive study of age-adjusted cancer mortality rates (1971-1978) for Iowa farmers and non-farmers did find a combined PMR for all types of cancer significantly lower for farmers. This lower rate was primarily explained by significantly lower rates for smoking-related cancers (esophagus, mouth other than a lip, and respiratory cancers). Mortality rates, however, were significantly elevated for cancer of the lip, stomach, multiple myeloma, leukemia, and prostate (Burmeister, 1981). Another case-control study, based on Iowa incidence cases, will analyze data to discern a possible cause-effect relationship of exposure to cattle, bovine lymphosarcoma virus, pesticides, and other factors in the rural agricultural environment (Donham, 1980). These incidence data from preliminary ecologic-epidemiologic studies have indicated elevated acute lymphocytic leukemia (ALL) rates strongly associated with male sex, rural residence, proximity to high cattle population densities, and to virus-infected dairy herds.

Projections

Concepts of causation in epidemiology cannot be based solely in mathematical analyses. Linkage between laboratory/clinical and epidemiologic information is needed to provide answers to disease etiology and prevention. Biological markers to identify individuals at risk or to identify origins of tumors will provide a rational basis for possible intervention, as further insights are gained into the pathophysiology of cancer.

Three recently funded studies will seek to document current implications of dietary intake of alcohol as a primary risk factor in oral and pharyngeal squamous cell carcinomas. Suggestions that farmers' health may not be as good as generally believed will be addressed in two new studies which will address the possible role of fertilizers, fungicide, and herbicides, as well as working environments associated with different types of farming.

Studies of multiple myeloma (MM) and chronic lymphocytic lymphoma (CLL) have been shown to be disorders of lymphocytes--cells which only recently have been recognized as playing a key role in the pathophysiology of many human disease processes. This "new immunology" has revolutionized concepts of body defenses against external invasion and neoplasia. Epidemiology can assist in elucidating these immunologic mechanisms by identification of risk factors and contributions to concepts of host/environment interactions mediated by the immune system (Weiss).

Descriptive epidemiologic studies to better characterize rare tumors will be encouraged. Until more information is obtained which describes the distribution among people, place, and times of various cancers, we will lack the necessary hypotheses based on actual clues obtained from data.

Methodology for assessing effects of low-dose exposure to various environmental exposures and contaminants remains an important research priority. Several studies are in the planning stages utilizing data from the large population-based cancer registries (e.g., SEER) for epidemiologic research.

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PREVENTIVE ONCOLOGY ACADEMIC AWARD SUMMARY

Description

The concept for the Preventive Oncology Academic Award (POAA) was presented to the National Cancer Advisory Board on October 5, 1979, and followed by a formal program announcement in the NIH Guide for Grants and Contracts (Volume 9, No. 1), January 3, 1980. Two receipt dates were declared: March 1, 1980 for funding July 1, 1980; and May 1, 1980 for funding July 1, 1981. For the future, one receipt date is anticipated. Of a total of 19 proposals received for the first deadline, eight were funded at the end of FY'80. It is likely that one of four received for the second deadline will be funded in late FY'81.

The POAA is not restricted to principal investigators representing a particular discipline, although epidemiology and biometry are strongly represented. Large-scale research efforts are not funded under the POAA. The funds are essentially "seed" money to be used to stimulate research, attract potentially capable researchers and clinicians while building an overall program in Preventive Oncology. Ongoing in-depth research and training related to cancer prevention receive support through mechanisms other than the POAA.

The research interests among the eight current POAAs reflects a diversity of approaches to cancer prevention. One awardee is developing research and teaching statistical models covering such topics as design and analysis of case-control and cohort studies; mathematical models for screening which illustrate the application of epidemiology and statistics to problems in cancer prevention based on research in industrial prevention, occupational exposures, Wilms' tumor, and the world-wide distribution of childhood malignancies. A computer program has been completed for analyses of case-control studies, which is in actual use by students and faculty.

Reflecting a totally different approach, another awardee is establishing a combined basic research and clinical application program in cancer prevention. This POAA has facilitated the development of an enzyme assay which may have the potential for use as a rapid routine clinical test for tumor responsiveness, i.e., 24-48 hours as opposed to current tests which take two weeks; and it has uncovered what may be the mechanism by which vitamin A inhibits neoplastic growth. This inhibition appears to be related to the ability of a ubiquitous enzyme, transglutaminase, to conjugate the primary amine putrescine to the enzyme orthinthine decarboxylase, with a resulting loss of activity.

The link between nutritional, occupational and environmental carcinogenesis is approached differently by several awardees. Genetic factors, screening, and clinical trials, as related to female reproductive cancers, are included in the research focus of four Preventive Oncology programs. Interest in the hormonal milieu of these cancers remains high, particularly as knowledge related to prevention is acquired.

The impetus given to the principal investigators' professional development has been encouraging. Several have noted that the POAA has increased recognition of the importance of preventive oncology and cooperation within the awardees'

institutions. A number of young medical and graduate students have requested electives to work within the preventive oncology programs which offer the opportunity for "hands-on" research experience, while being taught clinical oncology.

Projections

A meeting is anticipated in which the eight POAA awardees will share ideas, problems, and progress. Varying approaches, reflecting diverse research backgrounds, in areas of common interest should provide valuable means for stimulating new thrusts. The POAA to be funded from the second round of reviews seeks insight into primary cancer prevention by studying populations at low risk, e.g., epidemiology of cancer among Mormons. It will also seek to determine cancer incidence among megavitamin users and to examine cancer mortality among California physicians.

SMOKING AND HEALTH SUMMARY

Description

Since 1968, the National Cancer Institute's Smoking and Health Program (SHP) has been involved in efforts to understand and mitigate the deleterious effects of smoking on health. Close collaboration with the National Heart, Lung and Blood Institute and the U.S. Department of Agriculture has broadened the scope of research and contributed substantially to progress. Significant past SHP efforts have included development of practical techniques for making and testing less hazardous cigarettes, epidemiology studies seeking means for identifying groups of individuals at high risk to smoking related diseases, and pharmacological approaches to smoking cessation. Progress has been made to varying extents in each of these areas, the most significant being the evidence to date that low tar, low nicotine cigarette smoke is less harmful to experimental animals than high tar, high nicotine cigarette smoke. These findings are reflected in the current trend to low tar, low nicotine commercial cigarettes by the consumer. Current program emphasis is now focused on epidemiological and pharmacological aspects of the problem. In an attempt to attract more investigator-initiated research in these areas a Program Announcement was issued in January 1980. Response to date has been insufficient to develop a strong research base to better deal with this very important public health problem. Several new initiatives to accomplish this end have been outlined earlier in the Special Programs Branch Summary.

Research Accomplishments

The chemical analysis of whole smoke from commercial cigarettes for comparison with results from domestic epidemiology studies has continued. Forty brands of ultra-low "tar" cigarettes have been characterized for "tar", nicotine, carbon monoxide, hydrogen cyanide, oxides of nitrogen, and acrolein. A new, more effective approach for profiling vapor phase constituents of whole smoke has been developed. The approach is applicable to cigarettes of widely different tar deliveries, and initial data indicates that the organic vapor phase composition of the smokes are qualitatively similar.

In cooperative studies with the USDA, six types of experimental cigarettes, including bright and burley tobaccos, each with three different levels of nicotine as the only variable, are currently under tests. Although the long-term skin painting and short-term inhalation studies are still in progress, preliminary data indicates smoke from bright type tobacco is more active than smoke from burley tobacco. To date, there has been no correlation between nicotine levels and biological activity. This preliminary finding does not agree with previous studies, and if confirmed, raises the possibility that the reconstituted sheet process may have changed the combustibility of the tobacco components and thus the pyrolytic products.

The exposure phase of two bioassay studies using whole smoke inhalation in beagle dogs has been completed during the past year. The histopathology studies will require an additional four to six months for completion. Initial findings in one experiment indicate that there is a marked difference between beagles exposed to high tar/high nicotine cigarettes and those exposed to lower concentrations. The second experiment was designed to investigate the role of nicotine

and carbon monoxide in cigarette smoke as co-factors in the genesis of diet-induced atherosclerosis. Findings to date indicate that neither nicotine nor carbon monoxide per se appear to affect the development of atherosclerosis in the beagle fed a high cholestrol, low essential fatty acid diet. Epidemiology studies in six cities in five European countries are nearing completion. Data collection from histologically confirmed lung cancer cases along with controls has been completed. Data analysis by the Environmental Epidemiology Branch, National Cancer Institute, is now underway.

Data collection on a retrospective lung cancer case-control study in selected cities in the U.S. is continuing. This phase of the study is expected to be completed in early 1982. A prospective study, utilizing a self-administered questionnaire is underway at Kaiser Foundation Research Institute. This study is entering its third year and will involve approximately 80,000 ambulatory subscribers to a pre-paid medical care plan.

Studies have been initiated recently to examine the relationship between cigarette smoking and hepatocellular carcinoma. The effect of cigarette smoking on hepatocellular carcinoma risk will be estimated separately for cases who are negative for markers of hepatitis B virus infection and those who are positive for markers. Additional information will be obtained on each subject covering alcohol history, occupational history, and drug history. Work is continuing on tobacco-specific nitrosamines. The mechanism of carcinogenesis by nitrosonornicotine and the related nitrosaminoketone are being studied by standard methods of examining metabolites and binding to cellular macromolecules in target and non-target organs. Comparison will be made between metabolites found in experimental animals and those in the urine, blood, and saliva of smokers.

A recently awarded program project grant on tobacco-specific nitrosamines includes studies on nitrosodiethanolamine formation from MH-30, a major sucker-growth inhibitor used in tobacco and other vegatable crops, and the formation of areca-specific N-nitrosamines during betel nut chewing and its relation to oral cavity cancer.

Projections

The Smoking and Health Program is progressing toward its stated goals. Although many specific projects have been completed, there are still many areas in which work needs to be performed.

The histopathological studies on tissues from inhalation experiments will be completed. These results should yield valuable information on the relationship of whole cigarette smoke and nicotine concentrations to smoking-related diseases.

Epidemiological studies covering selected cities in the United States, six cities in five European countries and Cuba will be completed and evaluated. The vast amount of data relative to smoking habits, type of cigarette smoked, plus a broad medical data base, is expected to yield sufficient data to relate tobacco/cigarette characteristics to smoking habits and to disease incidence.

Identification of individuals at risk of developing tobacco-related disease continues to be of high priority. Continuation of the prospective epidemiologic study provides the potential for profiling characteristics which may contribute to susceptibility (or resistance) to smoking-related illness.

Animal studies investigating alterations in body fluids associated with tobacco use and subsequent disease may assist in identifying human smokers at unusually high risk of tobacco-related diseases. "State-of-the-art" workshops will be held during late 1981 to better define specific areas for further study which have potential for identifying these subgroups of the population.

LIST OF CONTRACTS

SMOKING AND HEALTH PROGRAM

Contract	Title	Page No.
Agriculture, Department of (YO1-CP-60209)	Toxicity of Tobacco and Smoke Components and Experimental Tobacco	1628
American Health Foundation (NO1-CP-05680)	Evaluation of Carcinogenic Agents in Cigarette Smoke	1629
American Health Foundation (NO1-CP-05684)	Epidemiology of Smoking-Related Diseases	1630
Borriston Research Laboratories (NO1-CP-05685)	Inhalation Bioassay of Cigarette Smoke in Dogs: High and Low Nicotine Content	1631
Energy, Department of (YO1-CP-60206)	Collection, Separation, and Elucidation of the Components of Cigarette Smoke and Smoke Condensate	1632
Enviro Control, Inc. (NO1-CP-55666)	Smoking and Health Prime Contract	1633
Hazleton Laboratories (NO1-CP-05679)	Effects of Nicotine and Carbon Monoxide in Cigarette Smoke on Atherogenesis	1633
International Agency for Research on Cancer (NO1-CP-05647)	Case Control Study of Lung Cancer in Cuban Women	1634
Kaiser Foundation Research Institute (NO1-CP-05681)	Surveillance of the Health Effects of Tobacco Products	1635
Southwest Foundation for Research and Education (NO1-CP-05683)	Inhalation Testing of Cigarette Smoke in Baboons	1636
Veterans Administration Medical Center (YO1-CP-80201)	Inhalation Bioassay of Cigarette Smoke in Male Beagle Dogs	1637

Contract	Title	Page No.
Western European Studies (NO1-CP-05641) (NO1-CP-05642) (NO1-CP-05643) (NO1-CP-05644) (NO1-CP-05645) (NO1-CP-05646)	An International Epidemiologic Study of the Relationship Between Tobacco Usage and Lung Cancer	1638

CONTRACT NARRATIVES

SMOKING AND HEALTH PROGRAM

AGRICULTURE, DEPARTMENT OF (YO1-CP-60209)

Title: Toxicity of Tobacco and Smoke Components and Experimental Tobacco.

Contractor's Project Director: Dr. T. C. Tso

Project Officer (NCI): Dr. Thomas B. Owen

Objectives: Determine the toxicity of tobacco and smoke components, and reduce the risk of smoking through elimination or reduction of harmful components by chemical, physical and biological means.

Major Findings: Six kinds of experimental cigarettes, including bright and burley tobaccos (each with three different levels of nicotine as the only variable, especially prepared by the USDA), were evaluated on smoke constituents, long-term skin painting and short-term inhalation. This study is still in progress. Preliminary data indicates that cigarette smoke condensate generated from bright type tobacco is more active than that from burley type tobacco. There is no significant difference so far among the three different nicotine levels.

Experimental tobacco produced by the USDA contains 20,000 times the Polonium-210 (^{210}Po) activity of normal tobacco. Experimental cigarettes were made with dilution of normal leaf (20:1), thus the experimental cigarettes used for inhalation study has 1,000 times higher activity than normal. Microdistribution of ^{210}Po in the lung of Fischer female rats was determined. Animals were sacrificed and autopsied both during and subsequent to a six-month smoking exposure period. Increase in activity in animal tissue was found with increase in time exposure. The air ways, separated from the whole lung tissue, showed track etch due to exposure to alpha activity. Complete data is not yet available because long exposure time (100-300 days) is required for these specimens. Alpha counting is quantitative and uniform from different sets. These results implicate ^{210}Po as one factor in smoking. The development of a technique to study alpha activity is significant in that it could be applied on a large area of the bronchial surface in smokers, ex-smokers and non-smokers. Also, the dosimetric calculations can be used to measure the alpha dose to the basal cells in bronchial epithelium.

A pilot plant study produced 600 lbs. of experimental materials which, if it demonstrates major differences after preliminary evaluation of chemical composition, may be used for experimental cigarette manufacture and smoke study.

A complete survey is underway on all components reported to be present in tobacco and tobacco smoke, which are estimated to be between 4,000 and 7,000 compounds. This information will be maintained in a word processor, indexed and published.

Significance to Biomedical Research and the Program of the Institute: Nicotine is one of the most, if not the most, important factor in the study of toxicity and pharmacology of smoking. The studies would provide basic information for

the evaluation of low-yield cigarettes. ^{210}Po may contribute to smoke toxicity and be particularly significant in passive smoking. Removal of soluble proteins and nitrates may reduce the formation of many undesirable smoke compounds, including No_x, quinolines, and nitrosamines. A survey of chemical compounds, and a readily available index would provide a valuable reference to all those concerned with the smoking and health problem.

Proposed Course: A. The nicotine study should be extended to metabolic and pyrolytic products of nicotine and their toxicological effects while examining the effects of leaf reconstitution on the combustion products of nicotine. This is important in evaluating the toxicology of low-yield cigarettes.

B. ^{210}Po studies should be expanded to include smokers, non-smokers, and ex-smokers to ascertain the distribution, level and changes of alpha activity in various organs.

C. Reduction of nitrogenous compounds is essential to reduce nitrosamines and many other harmful products related to smoke toxicology and pharmacology. Smoke and bioassay studies of these materials should be conducted.

D. A comprehensive index of currently known components in tobacco and tobacco smoke is very useful and needs to be diligently maintained as new compounds are reported.

Date Interagency Agreement Initiated: October 1, 1980.

Current Annual Level: $80,000.

AMERICAN HEALTH FOUNDATION (NO1-CP-05680) (Formerly ECI-SHP-75-109)

Title: Evaluation of Carcinogenic Agents in Cigarette Smoke.

Contractor's Project Director: Dr. Dietrich Hoffman

Project Officer (NCI): Dr. Thomas B. Owen

Objectives: The objective of this project is to identify tobacco and tobacco smoke constituents having adverse effects on health. This includes the isolation and identification of mutagens, tumor initiators, tumor promoters, cocarcinogens and organ-specific carcinogens from tobacco, tobacco additives and cigarette smoke.

Major Findings: Several tobacco-specific N-nitrosamines have been identified in tobacco and tobacco smoke. These are N'-nitrosonornicotine (NNN), 4-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK), and N'-nitrosoanatabine (NAtB). NNN and NNK induce tumors and carcinomas in the respiratory tracts of mice, rats and Syrian golden hamsters. These nitrosamines are also active as contact carcinogens in rats and hamsters. When given in drinking water, they induce tumors in the respiratory tract, mouth, and esophagus.

Significance to Biomedical Research and the Program of the Institute: The identification of toxic agents in smoke is necessary in order to determine what

steps can be taken to reduce or eliminate these compounds through manipulation of curing and processing practices currently in use.

Proposed Course: This contract has expired and the final report is in preparation.

Date Contract Initiated: September 9, 1980.

Current Annual Level: $232,096.

AMERICAN HEALTH FOUNDATION (NO1-CP-05684) (Formerly ECI-SHP-78-135)

Title: Epidemiology of Smoking-Related Diseases.

Contractor's Project Directors: Dr. Ernst L. Wynder
Dr. Margaret H. Mushinski

Project Officer (NCI): Dr. Thomas B. Owen

Objectives: The major objectives of this retrospective study include (1) measuring the effects of smoking cigarettes with varying levels of tar and nicotine on the risk of developing lung, bladder, pancreas and upper respiratory tract cancers plus first episode myocardial infarction; and (2) monitoring the use of the newer low tar cigarettes through hospital cases, their neighbors, and controls.

Major Findings: Between October 1980 and April 1981 close to 1,000 index cases and matched hospital controls were interviewed in select hospitals in New York, Philadelphia, Birmingham, Pittsburgh, Chicago, and San Francisco. A total of 417 persons living in the neighborhood of and matched to the interviewed index cases were also interviewed using the identical questionnaire.

Occupational level and smoking history were found to be significantly related ($p<.001$) among the white males interviewed during this reporting period. More professionals and men in technical occupations were found in the never smoke and ex-smoking categories; and more men employed in blue-collar positions were long-term smokers, smoked high tar filtered or non-filtered cigarettes, and began smoking earlier than men in white-collar positions.

Risk of developing lung or larynx cancer continues to be decreased among long-term (10+years) filter cigarette smokers when compared to long-term non-filter cigarette smokers. Risk of developing oral cavity cancer is associated with excessive alcohol consumption among cigarette smokers.

Preliminary analysis of male and female bladder cancer patients continues to support earlier findings that risk of this disease is not associated with saccharin or coffee consumption.

The smoking habits of the women in the study are becoming more similar to those of men. Proportionately more women choose filtered cigarettes, in general, and very low tar cigarettes (10 mg. tar), in particular, than men; and women begin the cigarette habit approximately three years later than their male counterparts.

Thus, the rate of lung cancer mortality in women will increase as more women who have long smoking histories reach the age in which cancer is most often found, but their incidence rates will not reach that of men.

Significance to Biomedical Research and the Program of the Institute: This epidemiological study is a necessary companion to the chemical and biological studies which try to identify the tumorigenic components in tobacco and tobacco smoke condensate and which measure the effect of new additives. Progress in the development of a less hazardous cigarette can only be measured in terms of decreased rates of human tobacco-related diseases. This study provides the Smoking and Health Program of the National Cancer Institute with data to (1) determine the nature of the less harmful cigarette and identify the constituents which may differentially affect various tobacco-related diseases, (2) encourage further development in the area of the less harmful cigarette, and (3) determine the effect of health education on smoking habits of a segment of the general population.

Proposed Course: Data collection and analyses of the following aspects of smoking and health will be continued: the tar content of cigarettes in relation to the risk of developing certain cancers and myocardial infarction, coffee consumption in relation to the risk of pancreas and bladder cancer, mouthwash use and the risk of oral cavity cancer, smoking habits of men and women in an effort to predict future disease patterns, and changes in quantity smoked after switching from a high tar to a low tar cigarette.

Date Contract Initiated: July 1, 1980.

Current Annual Level: $482,316.

BORRISTON RESEARCH LABORATORIES, INC. (NO1-CP-05685) (Formerly NO1-CP-55666-SA12)

Title: Inhalation Bioassay of Cigarette Smoke in Dogs: High and Low Nicotine Content.

Contractor's Project Director: Dr. V. Piccirillo

Project Officer (NCI): Dr. Thomas B. Owen

Objectives: The objective of this project is to develop techniques for using tracheostomized beagle dogs as a bioassay model for inhalation of whole cigarette smoke, and to determine varying physiological effects of high tar/nicotine and high tar/low nicotine cigarettes.

Major Findings: Initial findings indicate that the effects of different cigarettes with varying tar/nicotine content can be determined histologically. Several months will be required to complete histology and electron microscopy studies.

Significance to Biomedical Research and the Program of the Institute: A suitable animal model is needed for use in the bioassay of whole cigarette smoke. The increasing use by humans of the low tar/low nicotine cigarettes should be monitored by comparing results from epidemiology studies with laboratory studies in an animal model.

Proposed Course: All animals have been sacrificed. The pathology and histology studies will be completed and a final report prepared.

Date Contract Initiated: July 1, 1980.

Current Annual Level: $590,796.

ENERGY, DEPARTMENT OF (YO1-CP-60206)

Title: Collection, Separation, and Elucidation of the Components of Cigarette Smoke and Smoke Condensate.

Contractor's Project Directors: Dr. Michael R. Guerin
Dr. Roger A. Jenkins
Dr. Wayne H. Griest

Project Officer (NCI): Dr. Thomas B. Owen

Objectives: Activities carried out under this contract constitute physical and chemical resources to the National Cancer Institute and the Smoking and Health Program. This involves (a) providing quality-assured data on the deliveries of selected chemical constituents by commercial and experimental cigarettes, (b) providing validated methods for the quantitative determination of additional smoke constituents and for the assessment of smoke composition, (c) providing sampling and monitoring services to define exposures accompanying tobacco smoke inhalation exposure experiments, and (d) providing methods and data to establish the relationship of exposures and smoking conditions to the resulting dose of smoke constituents experienced by the smoker.

Major Findings: Part I: As part of the surveillance of U.S. commercial cigarettes, nearly forty brands of ultra-low "tar" cigarettes have been characterized for "tar," nicotine, carbon monoxide, hydrogen cyanide, oxides of nitrogen, and acrolein. Data indicated that smoke from non-tobacco cigarettes contained unexpectedly large quantities of hydrogen cyanide. Analysis of the smokes of selected U.S. cigarette brands marketed in Hong Kong suggests that they are of the same composition as their counterparts marketed in the United States. A new, more effective approach is applicable to cigarettes of widely different tar deliveries, and initial data indicates that the organic vapor phase composition of the smokes are qualitatively similar.

Part II: An instrumental monitor for the characterization of smoke exposure system performance has been developed and installed in a beagle dog inhalation study. Filter tip nicotine content as a measure of the amount of smoke generated during smoking may be applicable only to relatively high "tar" cigarettes. Normalization to constant urinary creatinine levels may be effective in reducing experimental variability in the apparent levels of nicotine and cotinine in smoke-exposed animals.

Significance to Biomedical Research and the Program of the Institute: The National Cancer Institute has assumed much of the responsibility for defining the carcinogenic potential of cigarette smoking and steps that might be taken to reduce that potential. Chemical studies provide the methods and data by which biological activity may be related to constituents of the smoke and

biological testing may be standardized. No other laboratory is engaged in general analytical chemistry and instrumental support of NCI tobacco smoke related studies.

Proposed Course: The contractor will continue to act as a tobacco smoke characterization and inhalation exposure facility for the National Cancer Institute by maintaining a core activity of analytical support services. Research activities are directed toward the development of a more cost-effective characterization of cigarette smokes and the identification and quantitative determination of smoke constituents and/or metabolites in physiological materials for tobacco smoke dosimetry in human beings.

Date Interagency Agreement Initiated: April 1, 1980.

Current Annual Level: $500,000.

ENVIRO CONTROL, INC. (NO1-CP-55666)

Title: Smoking and Health Prime Contract.

Contractor's Project Director: Dr. Allan P. Gray

Project Officer (NCI): Dr. Thomas B. Owen

Objectives: This contract became inactive, except for administrative close out, on July 1, 1980. Until that time the overall purpose was to facilitate progress toward the objectives of the Smoking and Health Program by providing essential scientific and administrative assistance as directed by the NCI in the following areas: (1) identification and development of new research efforts, (2) monitoring and evaluation of research projects, (3) technical and biostatistical analysis of data derived from subcontractors and interagency agreements, (4) dissemination of information by furnishing reports, documents, and papers to the NCI and through the NCI to the scientific community and general public, and (5) handling the purchase, storage, and distribution of tobacco supplies, cigarettes, equipment, and other materials.

Major Findings: The contractor performed the above described duties in a satisfactory manner during the tenure of the contract.

Significance to Biomedical Research and the Program of the Institute: The activities of the contractor were essential to the successful realization of the aims of the Smoking and Health Program during the period of time that the contract was active.

Date Contract Initiated: January 1, 1975.

Current Annual Level: 0.

HAZLETON LABORATORIES (NO1-CP-05679) (Formerly ECI-SHP-75-112)

Title: Effects of Nicotine and Carbon Monoxide in Cigarette Smoke on Atherogenesis.

Contractor's Project Director: Dr. William B. Coate

Project Officer (NCI): Dr. Thomas B. Owen

Objectives: The role of nicotine and carbon monoxide in cigarette smoke as co-factors in the genesis of diet-induced artherosclerosis in the beagle dog is being investigated.

Major Findings: Results to date indicate that nicotine, carbon monoxide, or cigarette smoke per se appear to affect the development of atherosclerosis in dogs on a high cholesterol, low essential fatty acid diet.

Significance to Biomedical Research and the Program of the Institute: Evidence is provided against the hypothesis that cigarette smoking is a factor in atherogenesis.

Proposed Course: Since October 1, 1980, all the effort in this contract has been on preparation of arterial tissue sample, histopathological evaluation, chemical analysis of tissues from the terminally (two-year) sacrificed dogs, and preparation of the final report. Histopathology was completed; and chemical analysis of aorta sections is near completion. The final report is scheduled for submission in May 1981.

Date Contract Initiated: April 7, 1980.

Current Annual Level: $278,160.

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (NO1-CP-05647)

Title: Case Control Study of Lung Cancer in Cuban Women.

Contractor's Project Director: Dr. Olga Joly

Project Officer (NCI): Dr. Thomas B. Owen

Objectives: Cuban women have the highest reported incidence of lung cancer of all comparable groups in the Americas; Cuban men have the third highest rates. This study is intended to identify specific factors associated with these high rates.

Major Findings: All personal interviews with lung cancer cases and controls have been completed. Data is currently being compiled and stored on computer tapes. Data analysis by the Field Studies and Statistics Program, DCCP, NCI will begin when the tapes are received from the contractor.

Significance to Biomedical Research and the Program of the Institute: The results of this study will be compared to those obtained from other Smoking and Health Program case-control studies to clarify the role of smoking and other factors in the development of lung cancer. These studies directly address two of the principal Smoking and Health Program objectives: (1) to identify individuals at high risk to tobacco-related diseases, and (2) to provide information for possible use by others in developing less hazardous cigarettes.

Proposed Course: Data will be analyzed by the Field Studies and Statistics Program, DCCP, NCI when computer tapes are submitted by the contractor. This contract will be allowed to expire on July 30, 1981.

Date Contract Initiated: July 1, 1980.

Current Annual Level: $45,483.

KAISER FOUNDATION RESEARCH INSTITUTE (NO1-CP-05681)

Title: Surveillance of the Health Effects of Tobacco Products.

Contractor's Project Director: Dr. Gary D. Friedman

Project Officer (NCI): Dr. Thomas B. Owen

Objectives: This project is a prospective, epidemiologic study of the health effects of possibly less hazardous cigarettes. The contractor continued to collect questionnaire information on past and current tobacco use, making progress toward their goal of enrolling into the study 80,000 ambulatory subscribers to a pre-paid medical care plan. The occurrence of hospitalized illness in members of this study population will eventually be ascertained in order to calculate the incidence rates of cancers and other serious illnesses in relation to the tar and nicotine content of the cigarette smoked at the time the questionnaire was completed. In order to obtain a ten percent random sample of the study population, information from the review of outpatient medical records is being retrieved. It will be used to study the relation of tar and nicotine level to the occurrence of conditions for which medical care is sought, but does not lead to hospitalization. A mailed, re-survey of a 10 percent random sample of last year's questionnaire respondents is in progress. This information will be used to assess the stability of smoking habits and cigarette brand preference in members of the study population.

As a separate project performed under this contract, a retrospective cohort analysis comparing mortality in persistent cigarette smokers with that in cigarette smokers who have quit smoking is being conducted.

Major Findings: The study questionnaires have been shown to be an accurate source of information on current smoking practices. Questionnaires may be the standard against which the physiologic measures of smoking, i.e., expired carbon monoxide and serum thiocyanate, should be judged.

Results to date have shown the risk of dying from coronary heart disease among persistent smokers was 2.2 times that of smokers who quit ($p = 0.004$), even after adjustment for baseline difference in cardiovascular risk. Adjustment for the higher baseline risk of persistent smokers, who smoked more cigarettes and reported more cardiovascular symptoms, was more than compensated by adjusting for the lower baseline risk since they were thinner and drank more alcohol. After similar adjustment, the risk of death from any cause in persistent smokers was 1.6 times that in quitters ($p < .001$).

In an analysis of data from health questionnaires completed at the same time that the smoking questionnaires were completed, there was no significant

association of a history of peptic ulcer disease with tar or nicotine content of currently smoked cigarettes.

Analysis of smoking questionnaire data showed that the mean number of cigarettes smoked per day was higher in smokers of low yield cigarettes (tar < 1.0 mg. per cigarette) than in smokers of high yield cigarettes at all ages and in both sexes. No differences in mean duration of cigarette smoking or in age at starting to smoke were observed. These observations provide evidence for "compensation" in smokers of low-yield cigarettes.

Significance to Biomedical Research and the Program of the Institute: This study addresses two program goals: (1) identification of individuals at high risk to tobacco-related diseases and (2) identification of toxic constituents of smoking products.

Proposed Course: Continue the collection of data from human subjects through the self-administered questionnaire and analysis of same.

Date Contract Initiated: October 1, 1980.

Current Annual Level: $190,000.

SOUTHWEST FOUNDATION FOR RESEARCH AND EDUCATION (NO1-CP-05683)

Title: Inhalation Testing of Cigarette Smoke in Baboons.

Contractor's Project Director: Dr. W. Rogers

Project Officer (NCI): Dr. Thomas B. Owen

Objectives: The objectives of this project are to (1) demonstrate that the baboon can serve as a surrogate cigarette smoker analogous to the human and (2) collect physiological data on animals as they smoke various types of cigarettes. Feasibility of this animal for a bioassay model should be determined.

Major Findings: The project has (1) developed the necessary instrumentation, training techniques, and dosimetry methods; (2) trained 13 animals, plus five controls, to smoke cigarettes in a human-like fashion using either a single-stage inhalation procedure or a two-stage inhalation procedure, which even more closely simulates the performance of many humans who inhale cigarette smoke; (3) demonstrated that the smoking behavior of the baboons can be controlled by operant conditioning techniques for periods of years with the animals smoking over two packs a day; (4) shown that the baboons will smoke a least 15 widely different types of cigarettes; (5) found that baboons develop carbon monoxide, thiocyanate, nicotine and cotinine levels comparable to those of human smokers; (6) measure smoking puff profiles in baboons finding puff volumes, pressure, and durations similar to those of human smokers; (7) studied the relationships between smoking behavior and cigarette type with absorption and clearance of carbon monoxide, thiocyanate, nicotine and cotinine in blood and urine; (8) found that although the cigarette smoking baboon is like the human in its preference for high nicotine content cigarettes, the baboon does not appear to smoke cigarettes without an extrinsic reinforcer; (9) demonstrated deposition

of significant amounts of particulate matter in the alveoli using ^{14}C-dotriacontane recovered by bronchoalveolar lavage; (10) observed various morphological, physiological, and biochemical changes, including elevations in aryl hyrocarbon hydroxylase (AAH) activity which were correlated with duration of smoking and degree of inhalation, in alveolar macrophages recovered by bronchoalveolar lavage; (11) demonstrated that, although most pulmonary function characteristics of the smoking animals were not different from normal, the smoking animals do show greater bronchoconstriction in response to methacholine aerosol challenge; (12) observed that nicotine aerosol reduces, or blocks, the bronchoconstricting effects of methacholine; (13) demonstrated that baboons will inhale 200 mg. of nicotine in aerosol form per day, producing blood and urine nictoine and cotinine levels five to ten times higher than those reported for human cigarette smokers; (14) found that the baboons will titrate their nicotine aerosol intake in response to manipulations of the pH of the aerosol solution and that, under some conditions, the animals prefer nicotine aerosol to cigarette smoke; and (15) observed decreases in platelet factor IV and increases in platelet factor VIII, both signs of arterial injury, in smoking animals.

Significance to Biomedical Research and the Program of the Institute: Development of an animal model whose physiology and smoking behavior closely resemble that of the human would be an important bioassay method. It would enable the study of experimental cigarettes in vivo, and would allow the study of possible health effects of cigarette smoking in an integrated experimental program.

Proposed Course: The contract has been completed and a final report submitted.

Date Contract Initiated: November 1, 1980.

Current Annual Level: $64,864.

VETERANS ADMINISTRATION MEDICAL CENTER (YO1-CP-80201)

Title: Inhalation Bioassay of Cigarette Smoke in Male Beagle Dogs.

Contractor's Project Director: Dr. Oscar Auerbach

Project Officer (NCI): Dr. Thomas B. Owen

Objectives: The objective of this study is to determine the extent of changes in the tracheobronchial tree, lung parenchyma and other organs of the body produced by smoking cigarettes of varying tar and nicotine content, whether these changes are less when cigarettes of progressively lower levels of tar and nicotine are smoked and whether there is a point to which tar and nicotine levels can be reduced so that only minimal changes result.

Major Findings: A preliminary review of findings in the previous VA-NCI study (Contract YO1-CP-40205), and findings in dogs from the Borriston Research Laboratories smoke inhalation study, revealed changes in the form of pulmonary emphysema and basal cell hyperplasia to be far less in the dogs in these studies than in the first and second smoking dog studies conducted in this laboratory in the mid-1960's using the unfiltered Camel and filtered Kent cigarettes, respectively. The more recent studies do show differences in the degree of

changes among the various groups of dogs with the least changes occurring in the groups smoking cigarettes of lower tar and nicotine content, and a progressive increase in these changes in the groups smoking cigarettes of higher tar and nicotine content.

Significance to Biomedical Research and the Program of the Institute: The endpoint of this study is not the production of lung cancer, but rather the study of developing pathology in various organs of the experimental animals to determine the effects of cigarettes of varying tar and nicotine content in the development of pulmonary and cardiovascular diseases. The contractor is attempting to determine the point to which tar and nicotine levels can be reduced so that only minimal changes will result from smoking cigarettes.

Proposed Course: The 115 male beagle dogs in the study have undergone pre-smoking electrocardiograms. Initial and interval blood samples have been drawn for hematology, clinical chemistry, protein electrophoresis, platelet aggregation, carboxyhemoglobin and blood nicotine levels.

The dogs have been divided into three groups of 30 each, using one group with each of the three types of cigarettes: A-1 (high tar/nicotine), A-2 (medium tar/nicotine) and A-3 (low tar/nicotine). ADL-II smoking machine is being used. A fourth group of 18 dogs serve as sham-smoked controls. A fifth group of seven dogs are being smoked using the A-1 cigarette on the Kinman model machine which was used in the first and second smoking dog studies. Animals will be sacrificed at 600 days of smoking.

Evaluation of the effects of the three types of cigarettes will be based upon the histologic examination of the lungs, tracheobronchial tree, cardiovascular system and all tissue specimens of the body systems of the dogs as deemed feasible by the Principal Investigator.

Date Contract Initiated: October 1, 1980.

Current Annual Level: $560,000.

WESTERN EUROPEAN STUDIES

Title: An International Epidemiologic Study of the Relationship Betweeen Tobacco Usage and Lung Cancer.

Contractor's Project Directors:

Dr. M. Kunze	(NO1-CP-05641)
Dr. R. Flamant	(NO1-CP-05642)
Dr. R. Schmahl	(NO1-CP-05643)
Dr. F. Berrino	(NO1-CP-05644)
Dr. G. Visco	(NO1-CP-05645)
Dr. C. Gillis	(NO1-CP-05646)

Project Officer (NCI): Dr. Thomas B. Owen

Objectives: The Smoking and Health Program has sponsored a case-control study of the relationships between tobacco usage and cancer incidence for several years in several U.S. cities. The purpose of the European studies is to increase

and substantiate our understanding of these relationships, particularly with respect to cancer of the lung. The range of tobacco products used, the variations in incidence of lung cancer, the differences in smoking habits, and the diversity of socioeconomic, demographic and occupational characteristics of the European nations provide a promising epidemiologic setting for comparisons with the U.S. experience.

A retrospective case-control design is used, based on the U.S. protocol. Subjects are selected from several hospitals in each country. Cases are hospitalized patients with a histopathologically confirmed diagnosis of lung cancer. Two controls are matched to each case by sex, race, age and hospital status. A detailed questionnaire is administered by a trained interviewer, covering, among other topics, tobacco habits and socioeconomic, demographic, and occupational background.

Major Findings: During the past year, interviews have been completed on lung cancer cases with matched controls as follows:

	Lung Cancer Cases	
	Male	Female
Rome	350	150
Scotland	100	150
Germany	260	40
Austria	300	100
Paris	300	50
Milan	350	50

The questionnaires are being edited at the present time. All data will be stored on tape and sent to the National Cancer Institute.

Significance to Biomedical Research and the Program of the Institute: The results of this study will be compared with those obtained from other Smoking and Health Program case-control studies to clarify the role of smoking and other factors in the development of lung cancer. These studies address two Smoking and Health Program objectives: (1) to identify individuals at high risk to tobacco-related disease and (2) to provide information on smoking habits and patterns related to disease.

Proposed Course: When data has been received from all contractors, statistical analyses and epidemiologic interpretations will be performed by the Field Studies and Statistics Branch, DCCP, NCI.

Date Contract Initiated: May 1, 1980.

Current Annual Level:

(NO1-CP-05641)	$57,983
(NO1-CP-05642)	$85,154
(NO1-CP-05643)	$33,200
(NO1-CP-05644)	$44,115
(NO1-CP-05645)	$65,282
(NO1-CP-05646)	$45,000

SPECIAL PROGRAMS BRANCH

GRANTS ACTIVE DURING FY 81

BIOMETRY PROGRAM

Investigator/Institution/Grant Number	Title
BARRON, Bruce A. Columbia University 2 R01 CA 19145-06	Optimization of Screening for Cervix Cancer
BLOIS, Marsden S. University of California 5 R01 CA 26655-03	Natural Language Access to Clinical Data Bases
BROSS, Irwin D. Roswell Park Memorial Institute 5 R01 CA 27514-02	Program in Biometrics of Cancer Epidemiology
CECH, Irina University of Texas Health Science Center 5 R01 CA 24138-02	Relation of Cancer Roles and Source of Drinking Water
DUDEWICZ, Edward J. Ohio State University 5 R01 CA 26254-03	A Model for Medical Treatments Evaluation
ELSTON, Robert C. Louisiana State University 5 R01 CA 28198-02	Statistical Genetic Analysis for Cancer Families
KLOTZ, Jerome H. University of Wisconsin 2 R01 CA 18332-07	Statistical Problems in Clinical Cancer Research
KNUDSON, Alfred G. Jr. Institute for Cancer Research 5 R01 CA 22780-04	Biomathematical Approaches to Cancer
KOZIOL, James A. University of California, San Diego 5 R01 CA 26666-03	Topics in Biostatistics
LACHENBRUCH, Peter A.* University of Iowa 5 R01 CA 24089-03	Estimation of Prognosis Using SEER Data
MANTEL, Nathan* George Washington University 3 R01 CA 15686-05S1	Cancer Epidemiology: Statistical Methods

MIKÉ, Valerie Memorial Sloan-Kettering Cancer Center 1 R13 CA 29801-01	Conference on Biostatistics in Clinical Oncology
MILLER, Kenneth J. Rensselaer Polytechnic Institute 1 R01 CA 28924-01	Computer Assisted Analyses of Carcinogenicity
MOOLGAVKAR, Suresh H. Institute for Cancer Research 1 R01 CA 25588-02	Temporal Evolution of Cancer
MURTHY, Vrudhula K. Cedars-Sinai Medical Research Institute 3 R01 CA 21380-03S1	Comprehensive Models for Cancer Survival and Mortality
MYERS, George C. Duke University 5 R01 CA 23399-03	Certification of Cancer Related Deaths
NEEL, James V. University of Michigan 1 P01 CA 26803-01A1	Program Project: The Study of Human Mutation Rates
PAFFENBARGER, Ralph S. Stanford University 5 R01 CA 25264-03	Early Predictors of Site-Specific Cancers
PAGANO, Marcello Sidney Farber Cancer Institute 1 R01 CA 28066-02	Statistical Computing and Clinical Trials of Cancer
PHILLIPS, Roland L. Loma Linda University 2 R01 CA 14703-08	Epidemiology of Cancer in Adventists - A Low Risk Group
PIERCE, Donald A. Oregon State University 1 R01 CA 27532-02	Statistical Methodology for Response-Time Data
PUNNETT, Hope H. St. Christopher's Hospital for Children 5 R01 CA 19834-04	Genetic Constitution and Cancer Predisposition
RUBINOW, Sol I. Cornell University 5 R01 CA 20610-03	Kinetics of Normal and Neoplastic Hematopoietic Cells
SCHNEIDER, Robert University of California, Davis 2 R01 CA 14916-07A1	Animal Neoplasm Registry

SCHOENFELD, David A. Sidney Farber Cancer Center 5 R23 CA 25162-03	Regression Analyses Techniques for Cancer Research
SKOLNICK, Mark H. University of Utah 1 R01 CA 28854-01	Genetic Epidemiology of Cancer in Utah Genealogies
STRONG, Louise C. University of Texas 1 R01 CA 27925-01A1	Genetic Etiology and Consequences of Childhood Cancer
SWIFT, Michael R. University of North Carolina 2 R01 CA 14235-09A2	Neoplasia-Predisposing Genes of Man
TARTER, Michael E. West Coast Cancer Foundation 1 R01 CA 28142-01A1	Modern Functional Representation in Cancer Research
TSUTAKAWA, Robert K. University of Missouri 1 R01 CA 29765-01	Statistical Analysis of Cancer Mortality Rates
WEISS, Kenneth M. University of Texas Health Science Center 2 R01 CA 19311-05	Genetic Epidemiology of Cancer
WHITTEMORE, Alice S. Stanford University 5 R01 CA 23214-03	Effects of Multiple Exposures-Quantitative Aspects
ZELEN, Marvin Sidney Farber Cancer Institute 2 R01 CA 23415-04	Statistical Models of Biomedical Phenomena
ZIMMERMAN, Stuart O. M.D. Anderson Hospital and Tumor Institute 2 R01 CA 11430-16	Biomathematics and Computing in a Cancer Institute
ZIPPIN, Calvin* University of California, San Francisco 5 R13 CA 23655-03	Conferences for Personnel of Cancer Data Programs

*Grants Active During FY 81 but Funded with Previous Year's Funds.

SPECIAL PROGRAMS BRANCH

GRANTS ACTIVE DURING FY 81

DIET/NUTRITION PROGRAM

Investigator/Institution/Grant Number	Title
BIRT, Diane F. University of Nebraska 5 ROl CA 24549-03	Influence of Dietary Selenium on Pancreatic Cancer
CAMPBELL, T. Colin Cornell University 5 POl CA 26755-02	Nutrition and Cancer
CHA, Young-Nam Johns Hopkins University 5 ROl CA 27594-02	Mechanism of Antimutagenesis by Anticarcinogens
CORWIN, Laurence M. Boston University 5 ROl CA 26604-03	Effect of Vitamin E and Lipids on Tumorigenicity
DAO, Thomas L. Roswell Park Memorial Institute 1 ROl CA 26597-02	Dietary Fat and Mammary Carcinogenesis
DE ROSA, Guglielmo University of Louisville 5 ROl CA 25602-03	Dietary Modifications, Exercise and Tumor Growth
DRAPER, Harold H. Univesity of Guelph 5 ROl CA 28242-02	Toxicity and Metabolism of Malondialdehyde
GERSHOFF, Stanley N.* Tufts University 5 ROl CA 23714-03	Effect of Diet on Vesical Tumor and Stone Formation
GRAHAM, Saxon State University of New York 2 POl CA 11535-11	Social Epidemiology and Control of Cancer
GRAY, James I. Michigan State University 5 ROl CA 26576-02	Formation of N-Nitroso Compounds in Processed Food
HAMILTON, Stanley R. Johns Hopkins University 1 ROl CA 29714-01	Role of Beer and Ethanol in Experimental Colon Cancer

HAWRYLEWICZ, Ervin J. Mercy Hospital and Medical Center 5 R01 CA 26547-02	Effect of Diet on the Hypothalamus and Breast Tumors
HEINIGER, Hans-Jorg Jackson Laboratory 5 R01 CA 19305-05	Cholesterol in Normal and Malignant Lymphocytes
HSIEH, Dennis P. University of California, Davis 5 R01 CA 27426-02	Comparative Toxicology of Carcinogenic Mycotoxins
IP, Clement C. Roswell Park Memorial Institute 5 R01 CA 27706-02	Selenium Supplement and Dietary Fat in Breast Cancer
JANGHORBANI, Morteza Massachusetts Institute of Technology 5 R01 CA 27917-02	Dietary Bioavailability of Selenium in Man
KOLONEL, Laurence N University of Hawaii 5 R01 CA 26515-03	Case-Control Study of Lung Cancer and Dietary Vitamin A
LE MAISTRE, Charles A. University of Texas 1 R13 CA 28905-01	1981 Annual Symposium on Fundamental Cancer Research
LYON, Joseph L.* University of Utah 5 R01 CA 21007-03	Diet and Colon Cancer in a Low Risk Population
MACKENZIE, Cosmo G. University of Colorado 5 R01 CA 27861-02	A Nutritional Control of Cancer
MEYSKENS, Frank L. University of Arizona 5 P01 CA 27502-02	Vitamin A Program Project
MILNER, John A. University of Illinois 1 R01 CA 29462-01	Dietary Arginine and Tumor Growth and Development
MUSEY, Paul I. Emory University 5 R01 CA 24616-03	The Effect of Diet on Estrogen Biosynthesis and Metabolism
NEWBERNE, Paul M. Massachusetts Institute of Technology 5 R01 CA 25382-03	Zinc, Nitrosamine, and Esophageal Cancer

NEWBERNE, Paul M. Massachusetts Institute of Technology 5 ROl CA 26917-02	Dietary Fat in Colon Carcinogenesis
PARIZA, Michael W. University of Wisconsin 1 ROl CA 29618-01	Structure and Origin of Mutagens in Fried Beef
PAWLOWSKI, Norman E. Oregon State University 2 ROl CA 25766-03	Mechanisms for Biological Activity of Cyclopropenes
PETHICA, Brian A. Clarkson College of Technology 5 ROl CA 26379-03	Dietary Fiber--the Physical Chemistry of Lignins
ROEBUCK, Bill D. Dartmouth College 5 ROl CA 26594-02	Modulation of Pancreatic Carcinogenesis by Diet
ROGERS, Adrianne E. Massachusetts Institute of Technology 3 ROl CA 25538-03	Dietary Fat, Prolactin and Mammary Cancer
ROSS, Morris H. Institute for Cancer Research 5 ROl CA 16442-07	Regulation of Tumor Susceptibility
RUDOLPH, Frederick B. Rice University 2 ROl CA 14030-09	Regulation of Metabolism by Purine Interconversions
SARKAR, Nurul H. Sloan-Kettering Institute for Cancer Research 5 ROl CA 25679-02	Effect of Diet on Murine Mammary Tumorigenesis
SCANLAN, Richard A. Oregon State University 2 ROl CA 25002-11	Nitrosamines in Foods
SELIVONCHICK, Daniel P. Oregon State University 1 ROl CA 30087-01	Membrane Protein Composition: Cyclopropenoid Fatty Acid
SHINOZUKA, Hisashi University of Pittsburgh 5 ROl CA 26556-02	Diet Modification and Promotion of Liver Carcinogenesis
SIDRANSKY, Herschel George Washington University 5 ROl CA 26557-02	Nutritional Influence on Chemical Carcinogenesis

SINNHUBER, Russell O. Oregon State University 5 R01 CA 20990-04	Protein Effects of Aflatoxin Carcinogenesis in Trout
SMITH, George S. University of California, Los Angeles 5 R01 CA 26164-02	Dietary Restriction, Cancer and Immune Functions
SPEIZER, Frank E. Channing Laboratory 5 R01 CA 26560-02	Prospective Study of Diet and Cancer in Women
THOMPSON, Henry J. University of New Hampshire 5 R01 CA 28109-02	Nutrition and Mammary Carcinogenesis
TROLL, Walter New York University Medical Center 5 R01 CA 16060-10	Inhibition of Tumor Promotion by Protease Inhibitors
VISEK, Willard J.* University of Illinois 5 R01 CA 22326-03	Dietary Protein--Fat Interactions in DMBA Mammary Cancer
VISEK, Willard J. University of Illinois 5 R01 CA 28629-02	Hormones, Dietary Fat and Mammary Carcinogenesis
WADE, Adelbert E. University of Georgia 5 R01 CA 29583-02	Effect of Dietary Fat Type on Chemical Carcinogenesis
WARREN, Guylyn R. Montana State University 5 R01 CA 26647-02	Mutagenic/Carcinogenic Agents in Body Fluids of Children
WEISBURGER, John H. American Health Foundation 1 P01 CA 29602-01	Nutritional Carcinogenesis
WEST, Dee W. University of Utah 5 R01 CA 25580-02	Diet and Colon Cancer in Man: The Effects of Fiber

*Grants Active During FY 81 but Funded with Previous Year's Funds.

SPECIAL PROGRAMS BRANCH

GRANTS ACTIVE DURING FY 81

EPIDEMIOLOGY PROGRAM

Investigator/Institution/Grant Number	Title
ALFORD, Charles A.* University of Alabama 5 R01 CA 16424-05	Herpes Virus Infections in High Risk Cancer Groups
AMSEL, Jonathan University of Pennsylvania 2 R01 CA 23544-03	Case-Control Study of Malignant Melanoma
ANDERSON, Henry A.* Mt. Sinai School of Medicine 5 R01 CA 22792-03	Cancer Among Household Contacts of Asbestos Workers
BEASLEY, R. Palmer University of Washington 5 R01 CA 25327-03	Hepatocellular Carcinoma Risk in Hepatitis B Carriers
BLUMBERG, Baruch Institute for Cancer Research 5 P01 CA 06551-18	Cancer Clinical Research at the Fox Chase Center
BURMEISTER, Leon F.* University of Iowa 5 R01 CA 24302-02	Farming as an Occupational Risk for Cancer
COMSTOCK, George W. Johns Hopkins University 5 R01 CA 24758-03	Cancer Studies in Washington County, Maryland
DAVIS, Scott Fred Hutchinson Cancer Center 1 R23 CA 29395-01	A Case-Control Study of Hodgkin's Disease
DELAFRESNAYE, John F. International Union Against Cancer 2 R13 CA 05096-20	Program of the International Union Against Cancer
DODD, Roger American Red Cross 1 R01 CA 31002-01	Hepatitis B Surface Antigen as a Risk for Hepatocellular Carcinoma
DONHAM, Kelley J. University of Iowa 1 R01 CA 28626-01	The Epidemiology of Leukemia in Rural Iowa

EVANS, Alfred S. Yale University 5 R01 CA 12952-07	Immunology of Persistent Infections with EB Virus
FIALKOW, Philip J. University of Washington 2 R01 CA 16448-07	Human Cancer--Origin and Genetic Markers
FISCHMAN, Harvey R. Johns Hopkins University 1 R01 CA/OH 30410-01	Cancer Mortality Among Workers in the Meat Industry
GREENWALD, Peter* New York State Dept. of Health 5 R01 CA 20502-04	Epidemiologic Study of Ovarian Cancer
GREENWALD, Peter New York State Dept. of Health 5 R01 CA 24367-03	Endometrial Cancer Epidemiology and Control
GRUFFERMAN, Seymour* Duke University 2 R01 CA 22104-04	The Epidemiology of Multiple Myeloma
HENDERSON, Brian E. University of Southern California 5 P01 CA 17054-06	Cancer Center Epidemiology and Biostatistics Support
HENDERSON, Brian E.* University of Southern California 1 R13 CA 27751-01	Epidemiology and Cancer Registries in the Pacific Basin
HERBST, Arthur L. University of Chicago 5 R01 CA 20084-05	Exogenous Maternal Hormones and Cancer in Daughters
HINDS, M. Ward University of Hawaii 1 R01 CA 30119-01	A 50,000 Member Cohort Study of Alcohol and Cancer
HOCHBERG, Fred H. Massachusetts General Hospital 2 R01 CA 22533-04	Epidemiology of Brain Tumors
HUTCHISON, George B. Harvard University 2 R01 CA 22849-04	Second Cancers in Patients with Hodgkin's Disease
HUTCHISON, George B. Harvard University 5 R01 CA 24209-03	An Epidemiologic Study of Cancer of the Ovary

JANERICH, Dwight T. New York State Dept. of Health 5 ROI CA 19564-03	Cancer in Parents of Congenitally Defective Children
JOHNSON, Carl J. Jefferson County Health Dept. 1 ROI CA 25729-03	Evaluation of Low-Level Plutonium and Cancer
KERSEY, John H.* University of Minnesota 5 ROI CA 18083-03	Epidemiology of Cancer in Immunodeficiency Families
KESSLER, Irving I.* University of Maryland 3 ROI CA 25018-02S1	A Prospective Study of Cancer Risk in Tuberculosis
KESSLER, Irving I. University of Maryland 5 ROI CA 25019-04	Male Role in Cervical Cancer
KESSLER, Irving I.* University of Maryland 5 ROI CA 25020-02	A Prospective Study of Lymphoid Tissues in Neoplasia
KULLER, Louis H.* University of Pittsburgh 5 ROI CA 24739-03	Relationshiop between Cholecystectomy and Ascending Colon
KURLAND, Leonard T. Mayo Foundation 5 ROI CA 25441-03	Study of Males Exposed in Utero to Diethylstilbestrol
LOPEZ-S, Arthur* Louisiana State University 5 ROI CA 23205-03	Lung Cancer and Vitamin A
LYNCH, Henry T. Creighton University 1 ROI CA 27831-02	Epidemiologic-Biologic Study of Colon Cancer Families
MACK, Thomas M. University of Southern California 5 ROI CA 23927-03	Case-Control Study of Malignant Melanoma
MACMAHON, Brian Harvard University 5 POI CA 06373-20	Cancer Epidemiology and Prevention Research Center
MACMAHON, Brian Harvard University 1 ROI CA 29723-01	An Epidemiological Study of Renal Adenocarcinoma

MATANOSKI, Genevieve M. Johns Hopkins University 1 R01 CA 24606-01	Cancer Risks in Virologists
MASHBERG, Arthur V.A. Medical Center, New Jersey Medical School 1 R01 CA 29214-01	Role of Alcohol as Primary Risk Factor in Oral Cancer
MEADOWS, Anna Children's Hospital of Philadelphia 1 R01 CA 29275-02	Heredity and Environment in Childhood Cancer
MORRISON, Alan S. Harvard University 5 R01 CA 24797-02	Breast Parenchymal Patterns and Breast Cancer Risk
MORTIMER, Edward A. Case Western Reserve University 1 R01 CA 26252-01	Epidemiology of Ovarian Carcinoma
MOSS, Andrew R. Northern California Cancer Program 1 R01 CA 27752-01A1	Testicular Cancer and Prenatal DES Exposure
NASCA, Philip C. New York State Dept. of Health 5 R01 CA 26194-02	Epidemiologic Study of Childhood Gliomas
PENDERGRASS, Thomas W. Children's Orthopedic Hospital and Medical Center 5 R23 CA 24186-03	Environmental Exposures and Childhood Leukemia
PRESTON-MARTIN, Susan University of Southern California 1 R01 CA 28215-02	Epidemiology of Tumors of the Parotid Gland
REEVES, William C. Gorgas Memorial Institute of Tropical and Preventive Medicine 5 R01 CA 25419-03	Cervical Cancer Epidemiology in Panama
ROSS, Ronald K.* University of Southern California 3 R01 CA 24082-02S1	Immunoblastic Lymphadenopathy and Histiocytic Lymphoma
ROSS, Ronald K. University of Southern California 5 R01 CA 25669-02	Epidemiology of Cancer of the Renal Pelvis and Ureters
ROTHMAN, Kenneth J. Harvard University 1 R01 CA 29666-01	Case-Control Study of Laryngeal-Hypopharyngeal Cancer

SAMET, Jonathan M. University of New Mexico 5 ROI CA 27187-02	Lung Cancer Etiology in New Mexico's Hispanics and Anglos
SCHOTTENFELD, David* Memorial Hospital for Cancer and Allied Diseases 5 ROI CA 17028-04	Cancer Risk After Irradiation and/or Chemotherapy
SPEIZER, Frank E. Peter Bent Brigham Hospital 5 ROI CA 23645-05	A Prospective Cohort for Risks in Breast Cancer
STARK, Alice New York State Dept. of Health 1 R23 CA 29713-01	Cancer Incidence and Death from All Causes in Farmers
SZKLO, Moyses Johns Hopkins University 5 ROI CA 24757-03	Epidemiology of Aplastic Anemia in Baltimore
SZKLO, Moyses Johns Hopkins University 5 ROI CA 26500-03	Epidemiological and HLA Study of Leukemia
WALLACE, Robert B. University of Iowa 2 ROI CA 15104-08	Anovulation and Epidemiology of Hormone-Responsive Tumors
WALLACE, Robert B. University of Iowa 2 ROI CA 15104-08S1	Anovulation and Epidemiology of Hormone-Responsive Tumors
WEISS, Noel S. Fred Hutchinson Cancer Research Center 5 ROI CA 23350-04	Epidemiology of Myeloma and Lymphocytic Leukemia
WYSHAK, Grace Harvard University 1 ROI CA 25623-02	Cancer Incidence in Mothers of Dizygotic Twins

*Grants Active During FY 81 but Funded with Previous Year's Funds.

SPECIAL PROGRAMS BRANCH

GRANTS ACTIVE DURING FY 81

PREVENTIVE ONCOLOGY ACADEMIC AWARD PROGRAM

Investigator/Institution/Grant Number	Title
BRESLOW, Norman E. University of Washington 5 K07 CA 00723-02	Preventive Oncology Academic Award
ENSTROM, James E. University of California, Los Angeles 1 K07 CA 00748-01	Preventive Oncology Academic Award
GRUFFERMAN, Seymour Duke University Medical Center 5 K07 CA 00726-02	Preventive Oncology Academic Award
LOVE, Richard R. University of Wisconsin 5 K07 CA 00721-02	Preventive Oncology Academic Award
RUDNICK, Seth A. University of North Carolina 5 K07 CA 00722-02	Preventive Oncology Academic Award
RUSSELL, Diane H. University of Arizona Health Sciences Center 5 K07 CA 00732-02	Preventive Oncology Academic Award
SCHOTTENFELD, David Memorial Hospital for Cancer 5 K07 CA 00727-02	Preventive Oncology Academic Award
STELLMAN, Jeanne M. Columbia University 5 K07 CA 00730-02	Preventive Oncology Academic Award
YATES, Jerome W. University of Vermont 5 K07 CA 00743-02	Preventive Oncology Academic Award

SPECIAL PROGRAMS BRANCH

GRANTS ACTIVE DURING FY 81

SMOKING AND HEALTH PROGRAM

Investigator/Institution/Grant Number	Title
COLE, Philip University of Alabama 1 ROl CA 29968-01	Hepatocellular Carcinoma and Cigarette Smoking
HECHT, Stephen S. American Health Foundation 5 ROl CA 21393-05	Metabolism of the Carcinogen Nitrosonornicotine
HOFFMAN, Dietrich American Health Foundation 1 POl CA 29580-01	Experimental Tobacco Carcinogenesis
HOMBURGER, Freddy* Bio-Research Institute 1 ROl CA 25082-01	Skin Bioassay of Carcinogenesis in Syrian Hamsters

*Grants Active During FY 81 but Funded with Previous Year's Funds.